UNIVERSITY OF ILLINOIS
AT CHICAGO

Office of the Dean (MC 922)
School of Public Health
2121 West Taylor Street
Chicago, Illinois 60612-7260

5511 '00 MAR 22 A10:18

March 16, 2000

Food and Drug Administration
Department of Health and Human Services
Room 1061, 5630 Fishers Lane, HFA-305
Rockville, MD 20852

Re: Center for Food Safety "Petition Seeking the Withdrawal of the New Animal Drug Application Approval for Posilac – Recombinant Bovine Growth Hormone (rBGH)": **FDA Docket No. 98P-1194**

I would like to submit the following documentation in support of the above Petition.

1. Epstein, S.S. Letter and Report on "Potential Public Health Hazards of Biosynthetic Milk Hormones" to FDA Commissioner Young, July 19, 1989.
2. Epstein, S.S. "Potential Public Health Hazards of Biosynthetic Milk Hormones." International Journal of Health Services, 20(1):73-84, 1998. (A peer reviewed journal)
3. Epstein, S.S. "Questions and Answers on Synthetic Bovine Growth Hormones." International Journal of Health Services, 20(4):573-582, 1990.
4. Epstein, S.S. "A Literature Summary (1985-1999) on the Hazards of rBGH Milk." The Politics of Cancer Revisited, Appendix XII, p. 618-627, East Ridge Press, Fremont Center, NY.
5. European Commission, Report of the Scientific Committee on "Animal Welfare Aspects of the Use of Bovine Somatotrophin," March 10, 1999.
6. European Commission, Report of the Scientific Committee on "Public Health Aspects of the Use of Bovine Somatotrophin," March 15-16, 1999.

Respectfully submitted,

Samuel S. Epstein, M.D.
Professor of Environmental and Occupational Medicine
and
Chairman, The Cancer Prevention Coalition

98P-1194

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Phone (312) 996-6620 • Fax (312) 996-1374

THE
UNIVERSITY
OF
ILLINOIS
COLLEGE OF MEDICINE
AT CHICAGO

Health Resources Management (M/C 922)
School of Public Health West
Box 6998, Chicago, Illinois 60680
(312) 996-2237,2295

July 19, 1989

Frank E. Young, M. D.
Commissioner
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Dear Commissioner Young:

I am writing to you on an issue of critical national concern relating to the safety of U.S. agriculture, in general, and to serious potential threats to safety of milk and meat, in particular.

I enclose a report entitled, "Potential Public Health Hazards of Biosynthetic Milk Hormones". Apart from raising questions on their efficacy, the report documents growing evidence on adverse veterinary effects of these hormones whose significance has been minimized by the industry and apparently not adequately recognized by the FDA. More critically, the report raises a wide range of unresolved questions on the human safety of consumption of milk and meat from hormone treated cows.

These concerns are all the more pressing in view of the fact that over the last five years the general public has been consuming unlabelled milk and meat from hormone treated cattle, in view of the fact that the FDA has stated that such foods are safe, and in view of the fact that the FDA appears to be moving closer to approving these hormones.

I would appreciate a reply at your early convenience.

Sincerely yours,

Samuel S. Epstein, M. D.
Professor of Occupational
and Environmental Medicine

Enclosure

Copies to the attached

Copies to:

USDA Sec. C. Yeutter
W. Graham (Executive Office of the President)
Sen. T. Daschle
Sen. W. Fowler, Jr.
Sen. A. Gore, Jr.
Sen. H. Kohl
Sen. P. Leahy
Sen. H. Metzenbaum
Sen. P. Simon
Cong. J. Conyers, Jr.
Cong. C. Hatcher
Cong. E. Madigan
Cong. J. Scheuer
Cong. M. Synar
Cong. H. Waxman
Cong. T. Weiss
Wisconsin State Sen. Winkle
Wisconsin State Sen. Feingold

POTENTIAL PUBLIC HEALTH HAZARDS OF BIOSYNTHETIC MILK HORMONES

Samuel S. Epstein, M. D.
Professor Environmental and Occupational Medicine
School of Public Health, University of Illinois Medical Center, Chicago
President Rachel Carson Council Inc., Washington, D. C.

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I. INTRODUCTION

The use of biosynthetic milk hormones raises fundamental ethical, social and economic considerations, including the continued viability of the small family dairy farm. The expanding use of Bovine Growth Hormone (BGH)* and its methionyl analog (met-BGH)** also poses significant potential public health hazards which have not so far been investigated. These concerns are exacerbated by the virtual domination of BGH and met-BGH research by industry, by failure of the industries concerned to disclose their unpublished data, and by refusal to label milk and meat from cows treated with biosynthetic hormones and denial of consumers' rights to know. These concerns are further exacerbated by the abdication of regulatory responsibility by the FDA and USDA.

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* Manufactured by the Agricultural Chemicals Division of Elanco (Eli Lilly & Co.) in conjunction with Dow Chemical Co., and Upjohn Co.

** Manufactured by American Cyanamid Co. and Monsanto Co.

II. TRACK RECORD OF FDA AND USDA

FDA is responsible for approving the registration and use of animal drugs and issuing residue tolerances. Section 512 of the 1968 Animal Drug Amendments to the 1938 Federal Food Drug and Cosmetic Act (FFDCA) mandates FDA to require manufacturers submitting new animal drug applications to provide "a description of practical methods" for analysis and monitoring of drug residues in food. USDA is responsible for monitoring food animals and their products by FDA approved methods in order to detect and prevent the occurrence of illegal food residues.

The granting by FDA of an Investigation New Animal Drug (INAD) exemption for BGH and met-BGH on the basis of allegedly confidential data, and their allowing the sale of unlabelled hormonal milk and meat reflects the agency's relaxed view of its responsibilities. As stated in a September 1986 letter from the FDA Commissioner to Rep. Tony Coelho of the House Committee on Agriculture, -- "Sponsors have not been required to measure the increase of BST (BGH) in milk of treated cattle over that in milk from untreated cattle. Rather the safety of BST is based on the limited quantity of BST administered on a daily basis and the fact that BST is not biologically active in humans or other primates." Furthermore, in granting the INAD exemption, the FDA is in apparent violation of the 1968 FFDCA amendments which mandate that the Agency must have a "prescribed and approved" test method, which the industry is required to provide, for determining whether the drug is being improperly used with resulting illegal residues in food.

Of additional concern is the fact that FDA has inappropriately relied on standard toxicological protocols which are largely irrelevant for the safety evaluation of biosynthetic milk hormones. In fact, the only reported evidence of adverse effects has emerged from incidental findings in efficacy trials based on

Technical Advisory Document (TAD) protocols designed primarily for milk production trials. In particular, the agency has failed to require safety evaluation of milk from appropriate multilactational and multigenerational studies on a wide range of critical veterinary, let alone public health, concerns.

The conduct of the regulatory agencies with regard to milk hormones is consistent with their track record. As evidenced in an extensive series of Government Accounting Office investigations and Congressional hearings, USDA and FDA regulation is in near total disarray, aggravated by denials and coverups. A 1986 Congressional report concluded: "FDA has consistently disregarded its responsibility, -- repeatedly put what it perceives are interests of veterinarians and the livestock industry ahead of its legal obligation to protect consumers, -- jeopardizing the health and safety of consumers of meat, milk and poultry." (U.S. HR, 1985). Further illustrative is the April 1989 USDA proposal to end inspection of the nation's 6,300 meat and poultry processing plants, and instead to rely on voluntary compliance. The proposed plan, originally entitled "Discretionary Inspections" and then euphemistically renamed, "Improved Processing Inspection System", has met with a storm of criticism from sources including the American Meat Institute and major meat packers.

III. INDUSTRY CLAIMS ON MILK HORMONES

The industry claims, as exemplified in a recent promotional report (AHI, 1988), are highly misleading. It is claimed that the hormones increase milk yields by an average of 10-25%, that milk quality is unchanged, that increased hormone levels are not found in milk, that there are no adverse effects in treated cows, and that the biosynthetic hormones are safe as they are not biologically active in humans.

The AHI report quotes from a Cornell University milk hormone production trial to the effect that -- "it appeared that the cows were simply unaffected", and emphasizes that "subsequent studies at more than 20 universities confirm many of these observations." The report omits reference to the wide range of adverse effects noted in about half the limited number of met-BGH production trials (see Sec. IV), and makes no reference to met-BGH, except incidentally in an efficacy graph. Finally, the report makes no reference to the highly variable and inconsistent yields in the milk production trials.

Apart from misrepresentations, the industry claims are restrictedly based on small numbers of cows (7-10) per test group, reflecting TAD efficacy protocols in which adverse veterinary effects were only incidentally noted. Claims that increased hormone levels are not found in milk are suspect as they do not reflect anticipated dose-response relationships, and as they do not reflect increased plasma levels noted in several studies (see Sec. V).

The industry claims for the milk hormones are based on a complex of strategies. These misleadingly exaggerate efficacy, omit reference to documented adverse veterinary effects, and fail to undertake critical studies which could elicit information on adverse veterinary and public health effects. The past success of the industry strategies also reflects the unbalanced and indentured nature of in-house and academic research on milk hormones.

IV. ADVERSE VETERINARY EFFECTS

Available data on adverse veterinary effects in cows hyperstimulated by daily injections of BGH and met-BGH are sparse, and based on incidental findings in small scale milk production trials. The significance of these findings, to which no reference is made in industry promotional literature, is emphasized by the small size of the trial groups (7-10 cows). The gross statistical insensitivity of such trials has been recently emphasized. "At least 2,423 cows would be needed in each group to detect an increase in disease frequency from 5-10%, and at least 11,773 cows in each group for a change from 1-2% (Kronfeld, 1987). The importance of stress-related diseases associate with prolonged elevation in plasma levels of BGH has been strikingly confirmed in transgenic pigs in which there were "-- significant improvements in both daily weight gain and feed efficiency." However, these pigs also developed "-- a high incidence of gastric ulcers, arthritis, cardiomegaly, dermatitis and renal diseases." (Pursel et al, 1989)

1. Negative Energy Balance

Biosynthetic milk hormones induce a negative energy balance, similar to that in the rising phase of lactation, for some 8 weeks during which increased milk production is paralleled by "reduced total body fat", excessive tissue loss, and hypertrophy of foregut tissue (Brown et al, 1989). This sustained negative energy balance appears associated with increased stress, susceptibility to infectious disease and measurable changes in the composition of milk.

2. Increased Incidence of Infectious Diseases

In the Cyanamid-Pennsylvania met-BGH trial, mastitis developed in 4/8 cows at 12.5 mg/d and in 2/7 in 50 mg/d; high somatic cell counts were observed at all dosages in the Monsanto-Missouri trial, and at 25 mg/d in the Cyanamid-Missouri trial. Additionally, a high level of unspecified infectious disease was noted in 1/9 trials (Kronfeld, 1987).

3. Reduced Fertility

Evidence of reduced fertility was noted in 4/9 trials (Kronfeld, 1987; Mepham, 1989).

4. Heat Intolerance

This was noted at two dosage levels in 1/9 trials (Kronfeld, 1987). Such intolerance could pose particular problems for uses of biosynthetic hormones in tropical climates.

5. Changes in Nutritional Quality of Milk

Available data on the effects of hormones on the nutritional status and composition of milk, including protein sub-fractions, vitamins and minerals, are minimal. However, it is clear that the hormones induce a wide range of measurable changes in milk composition. Increased fat yields and concentrations have been noted (Bitman et al, 1984). Additionally, there is a statistically significant increase in long chain fatty acids and decrease in short chain fatty acids (Baer et al, 1979); this is associated with reduction in casein, in relation to both total and true protein, which is likely to decrease cheese yields. Such significant changes in the composition of milk in hormonally-treated cattle are becoming increasingly recognized (e.e. Mepham, 1989).

6. Questionable Efficacy of Milk Hormones

The adverse veterinary effects so far noted are not necessarily offset by improved milk production. Contrary to promotional claims, the effects of BGH and met-BGH on milk production are highly variable and inconsistent. In 9 met-BGH trials, outstanding responses were obtained in 2 herds and very poor responses in another 2 herds. "About one-third of all BST-treated herds would be predicted to fall between the consensus low limit of 10% more milk and my estimate of minus 1% based on the 9 trials." (Kronfeld, 1988). Burnout or lactational crash has been noted in hormone-treated cattle particularly at high dose levels, although no data are available on its incidence.

7. Other Growth Hormones in Milk

Apart from unresolved questions on incremental BGH levels and of any met-BGH levels in milk, Insulin-like Growth Factors (IGF-1), whose endogenous

production is stimulated by milk hormones, have been detected in milk of BGH-treated cows. Based on the very limited available data, levels in treated cows' milk appear to be sustained at high levels, similar to those found in untreated cows after the first week of lactation (Prosser, 1987, 1988). Additionally, the normal inverse relationship between endogenous growth hormone and blood insulin levels is disturbed following BGH treatment (Davis et al, 1987).

8. Misuse of Milk Hormones

Apart from concerns on overdosage of lactating cows, the use of BGH as a growth promoting hormone in calves and sheep has also been reported. Such misuses are all the more likely in view of the absence of practical and sensitive methods for detecting and monitoring hormonal levels in milk and meat. Also, the documented record of extensive misuse of growth promoting sex hormone animal drugs does not suggest that milk hormones will be handled any more responsibly.

9. Critical Data Gaps

It should be stressed that no information is available from large scale multilactational and multigenerational dose-response tests on a wide range of veterinary and related concerns. These include: milk production efficacy; alterations in detailed biochemical composition of milk, its nutritional quality, and its suitability for cheese production; alterations in reproduction and fertility; endocrinological effects; biochemical, endocrine and metabolic evidence of stress; stress-induced susceptibility to and increased incidence of viral infections, including bovine leukemia; increased levels in milk of antibiotics necessitated by increased bovine infections; allergenicity and immunogenicity of hormonal milk; response of hormone-treated cattle to vaccines; mobilization in milk of fat soluble carcinogens from depot fat by the sustained lipolytic action of milk hormones; and, identification and measurement in milk and meat of BGH and met-BGH, and of incremental levels of IGF-1 and other somatomedins.

V. POTENTIAL ADVERSE PUBLIC HEALTH EFFECTS

An editorial in a conservative British medical journal recently warned that before BGH can be considered commercially, "one would need to be completely reassured that the appropriate tests have been carried out thoroughly and professionally and that there is not the slightest hazard to human health." (The Lancet, 1988). In fact, the use of milk hormones poses serious risks of adverse public health effects that have not been adequately considered, in spite of continued strident industry assurances of safety. Apart from a wide range of information gaps that negate such assurances, there are some highly suggestive contrary data.

1. The Relationship of Biosynthetic to Natural Milk Hormones

The industry claims that BGH is "natural" are false. Both BGH and met-BGH are xenobiotics (Mepham, 1989).

The natural bovine hormone consists of 191 amino acid residues in linear sequence. The Elanco biosynthetic hormone, however, has a series of 8 additional amino acid residues, known as linker protein, at one end of the molecule (Brunner, 1988). In addition to such chemical differences, BGH is synthesized on a bacterial rather than a mammalian ribosome, and will thus have a different 3-dimensional structure and possibly different biological activities from natural BGH. The more potent met-BGH has an alien methionyl terminal residue. The FDA has recently admitted that biosynthetic milk hormones "are about 0.5 to 3% different in molecular structure" from the natural hormone (FDA, 1989).

2. The Biological Activity of Milk Hormones

The industry initially claimed that BGH was "species-specific" to cattle, and thus could not possibly have any effects in humans. However, BGH is now known to be active in a wide range of species including goats, pigs,

sheep, mice, and even fish. Accordingly, the industry has changed its position and now claims that BGH is "species-limited" (CIWF, 1988).

BGH derived from pituitary glands was shown in the 1950's to have "no effect on human growth, sexual development or well-being" (Monsanto, 1987). BGH is immunologically different from the human hormone, and differs structurally in some 30% of its amino acid residues. While BGH is inactive in all primates, it should be noted that human growth hormone is only active in humans when given in high (mg) doses. Moreover, no studies on humans have been conducted with biosynthetic milk hormones, especially the more potent met-BGH, which are chemically different from natural pituitary BGH. Furthermore, it was demonstrated some 30 years ago that proteolytic digests of natural BGH are biologically active in humans in whom they induce nitrogen retention (Forsham et al, 1958). Thus, biosynthetic milk hormones could be directly active in humans following absorption of novel peptides, formed during pasteurization or proteolytic digestion in the alimentary canal. Additionally, the intact hormone molecule could be absorbed into the blood from the digestive tract, particularly in newborn infants prior to closure time and in infants or adults with impaired protein digestion in diseases such as cystic fibrosis; absorption of intact protein molecules has been demonstrated in newborn babies and some adults (Mepham, 1989). The industry recently admitted that "some proteins are absorbed into the blood stream without being fully digested --." (Monsanto, 1987).

Industry claims that increased BGH milk levels are not found in dosed cows (AHI, 1988). In a recent publication purporting to confirm these claims, the upper range of levels in cows treated with 25 mg/day of BGH was more than 50% in excess of controls (Kennelly & deBoer, 1988). Furthermore, dose-response relationships for plasma BGH levels in the range of 5-30 ppb (ng/ml) have

been reported (Eppard et al, 1985). Up to 700% increased levels of plasma BGH have been reported following BGH dosing in late lactation (Peel et al, 1982); others have confirmed such elevations (e.g. Fronk et al, 1983). However, excess BGH levels have not been reported in milk assays by industry and its contractees. Clearly, milk of treated cows should be assayed by independent scientists using techniques which have yielded clear-cut results with plasma.

3. The Biological Activity of Growth Factors

There is a growing consensus that the mechanism of action of the pituitary growth hormone is via the induction of somatomedin growth factors, particularly IGF-1 (McBride et al, 1988). From all criteria, bovine and human IGF-1 appear identical (Honegger, 1986; McBride et al, 1988). Most of the specific activities of BGH, including milk production, gluconeogenesis, diabetogenesis, nitrogen retention, lipolysis, mitogenesis, adipose tissue and bone growth, are mediated through somatomedins. Moreover, mammary gland receptors for IGF-1 have been identified (Glimm et al, 1988).

Increased IGF-1 levels have been reported in goats milk following BGH treatment (Prosser, 1987). As subsequently briefly reported, high levels of IGF-1 are found in normal cows milk immediately after calving, falling to 1-5 ng/ml by 200 days (Prosser, 1988). However, levels induced by daily injections of BGH were sustained at 6-20 ng/ml. Thus, irrespective of possible activity in humans of BGH digestion products, mitogenic effects of BGH could be indirectly induced in humans by sustained incremental levels of IGF-1 and other somatomedins. Such effects could include premature growth stimulation in infants, gynaecomastia in young children, and breast cancer in adult females.

A recent publication insisting that BGH technology is sound, nevertheless warned (McBride et al, 1988). -- "Investigation of IGFs requires attention, particularly where animal health and food residues are concerned since they possess many biological activities and are immunologically and biologically similar among species. -- Some concerns arise as to the possibility of

abnormal levels of IGF-1 in the milk of BGH-treated cows and, with it, consumer health." Another publication warns. "The implications of IGF-1 in milk for the human infant cannot be determined until we know more about the activity and function of milk IGF-1 in the newborn. However, total growth factor activity in cow's milk, as assessed by a cell proliferation test in vitro which also detects components other than IGF-1, is not altered by bST treatment." (Prosser, 1988).

In addition to detailed studies on IGF-1 levels in milk of BGH-treated cows, the effects in humans of increased levels should be studied with priority, particularly since some consumers have already and unknowingly been exposed to BGH milk; this population at risk should be identified and subjected to long term surveillance. Systematic studies on IGFs should include dose-response in vitro investigations with human cells and tissues, and dose reponse studies in infant and adult primates, with a view to defining the effects of incremental IGF milk levels in humans.

4. Activity of Hormonally-induced Stressor Metabolites

The levels in milk of stressor metabolites, induced by BGH, met-BGH and somatomedins, such as epinephrines, catecholamines and cortisol, should be determined by sensitive and specific assays. The stressing action in humans of these metabolites should be investigated.

5. Infectivity of Hormonal Milk

The stressing effect in cows of BGH, met-BGH, and somatomedins may induce immunosuppression and activate latent viruses, such as Bovine Leukosis virus (BLV), and Bovine Immunodeficiency virus (BIV) which increases susceptibility to other infectious agents. Levels of such viruses in hormonally treated milk and their human infectivity should be investigated with particular reference to risks of immunosuppression and leukemia. The relationship between these viruses and the AIDS complex is of further concern.

6. Antibiotics in Hormonal Milk

The increased incidence of infectious diseases, which has been noted in efficacy trials and which is presumably stress-induced, is likely to result in increased antibiotic treatment and antibiotic levels in milk. Accordingly, the incidence of infectious diseases and of antibiotic milk levels should be investigated with particular reference to the risks of induction of antibiotic resistance in the general population.

7. Allergenicity of Hormonal Milk

The allergenic and immunogenic effects in humans of met-BGH in milk, and of novel peptides resulting from its pasteurization or digestion, should be investigated. It should be noted that there is substantial evidence on the high incidence of antibody development in humans treated with methionyl human growth hormone, rather than with the natural hormone (Eli Lilly & Co., 1987).

8. Fat-soluble Carcinogens in Hormonal Milk

The fat and milk of cattle are contaminated with a wide range of carcinogens including pesticides, such as heptachlor epoxide and dieldrin, and xenobiotics, such as PCBs and tetrachlorodibenzodioxin. The lipolytic effect of hormonal treatment is likely to mobilize carcinogens from body fat and increase their milk levels, a matter of particular concern to young infants. For these reasons, levels of fat soluble carcinogens in hormonal milk should be determined.

9. Nutritional Quality of Hormonal Milk

The nutritional quality of hormonal milk should be investigated in multilactational and multigenerational tests. As recently emphasized, such data "on detailed components of milk, e.g. casein fractions are not available" (Kennelly & deBoer, 1988). Available data, however demonstrate major increases in long chain saturated fatty acids relative to medium and short chain saturated fatty acids, and up to 27% higher fat levels in BGH milk (Bitman et al, 1984).

Dose-response relationships between milk fat and BGH have also been reported (Eppard et al, 1985).

10. Misuse of BGH and Met-BGH

In the event that registration should ever be granted to these biosynthetic hormones, there would be no practical method to prevent their extensive misuse, as well documented for sex growth hormones, or to detect and even monitor for such misuse. These hormones could then be administered at excessive dosages to lactating cows or as growth stimulants to calves, sheep and other cattle, increasing still further exposure of the general public to these highly potent biological agents.

VI. PUBLIC POLICY RECOMMENDATIONS

1. The manufacture, domestic sale and export, including foreign licensing agreements, of biosynthetic milk hormones should be banned immediately. This ban should remain effective until a wide range of concerns on public health, and veterinary, safety, have been posed and fully resolved.

2. The sale of milk, milk products and meat from hormone-treated cows should be embargoed immediately. To insure compliance, industry and its academic contractees must be required to immediately identify all treated cows and herds.

3. Attempts should be made to identify and place under long term medical surveillance all consumers, especially infants, who are at potential risk from having consumed BGH- and met-BGH-contaminated milk, milk products and meat.

4. The industry and its academic contractees must be required to immediately make full disclosure of all unpublished data and reports; claims for confidentiality must be legally preempted on the grounds of overriding concerns on public health and welfare.

5. The conduct of industry, and of its academic contractees, should be subject to Congressional investigation.

6. The conduct of the FDA in granting an INAD exemption for the testing of BGH and met-BGH in cows and approving sale of hormonal milk, in apparent violation of the 1968 FFDCA amendments, should be subject to legal challenge and Congressional investigation.

7. The industry must be required to develop and undertake multilactational and multigenerational dose-res-onse and other protocols appropriate for the investigation of potential adverse public health effects from hormonally contaminated milk, milk products and meat. Such research should be subject to ongoing independent review. These protocols must include: specific and

sensitive assays for BGH, met-BGH and somatomedins; investigation of the biological activity of these hormones and growth factors in milk; analysis of milk for stressor chemicals; investigation of the biological activity of such stressor chemicals at levels expected in hormonal milk; analysis of milk for antibiotics necessitated by treatment of stress-induced infections in lactating cows; analysis of milk for stress-induced or activated viral agents; analysis of milk for increased levels of fat soluble carcinogens mobilized by BGH or met-BGH; investigation of the allergenicity and immunogenicity of met-BGH, and of any derived novel peptides; investigation of the response to vaccines of treated cows; and detailed analysis of the nutritional quality of hormonal milk.

8. The industry must also be required to fund research in accordance with the approved protocols, which should be awarded, supervised and otherwise administered by a neutral independent intermediary such as the National Institutes of Health or the National Science Foundation.

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POTENTIAL PUBLIC HEALTH HAZARDS OF BIOSYNTHETIC MILK HORMONES

Samuel S. Epstein

The use of biosynthetic milk hormones raises fundamental ethical, social, and economic considerations, including the continued viability of the small family dairy farm and adverse veterinary effects. The past and expanding use of synthetic bovine growth hormone manufactured by the Agricultural Chemicals Division of Elanco (Eli Lilly and Co.) in conjunction with Dow Chemical Co. and Upjohn Co., and its methionyl analog, manufactured by American Cyanamid Co. and Monsanto Co., also poses significant potential public health hazards which have not so far been investigated. These concerns are exacerbated by the domination of synthetic hormone research by industry and its indentured academics, by failure of the industries concerned to disclose their unpublished data, by their manipulation of published data, and by refusal to label milk and meat from cows treated with biosynthetic hormones, and by denial of consumers' rights to know. These concerns are further exacerbated by the abdication of regulatory responsibility by the Food and Drug Administration and U.S. Department of Agriculture.

TRACK RECORD OF THE FOOD AND DRUG ADMINISTRATION AND THE U.S. DEPARTMENT OF AGRICULTURE

The Food and Drug Administration (FDA) is responsible for approving the registration and use of animal drugs and issuing residue tolerances. Section 512 of the 1968 Animal Drug Amendments to the 1938 Federal Food, Drug, and Cosmetic Act (FFDCA) mandates the FDA to require manufacturers submitting new animal drug applications to provide "a description of practical methods" for analysis and monitoring of drug residues in food. The U.S. Department of Agriculture (USDA) is responsible for monitoring food animals and their products by FDA-approved methods in order to detect and prevent the occurrence of illegal food residues.

The granting by the FDA of an Investigative New Animal Drug (INAD) exemption for the synthetic hormones on the basis of allegedly confidential data and their allowing the sale of unlabeled hormonal milk and meat reflects the agency's highly relaxed view of its responsibilities. As stated in a recent FDA Talk Paper, and elsewhere, sponsors have not been required to measure the increase of bovine growth hormone (BGH) in milk of treated cattle over that in milk from untreated cattle. Rather, the

This article is based in part on Testimony on Assembly Bill 200, "Relating to Bovine Growth Hormones," Wisconsin State Assembly Committee on Agriculture, State Capitol, Madison, September 6, 1989.

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that increased hormone levels are not found in milk, that there are no adverse reproductive or other effects in treated cows, and that the synthetic hormones are safe because they are not biologically active in humans. The Animal Health Institute report quotes from a milk hormone production trial conducted by Cornell University to the effect that "it appeared that the cows were simply unaffected," and emphasizes that "subsequent studies at more than 20 universities confirm many of these observations." The report omits reference to the wide range of adverse effects noted in about half the limited number of methionyl-BGH (met-BGH) production trials (see "Adverse Veterinary Effects") and makes no reference to met-BGH, except incidentally in an efficacy graph. Finally, the report makes no reference to the highly variable and inconsistent yields in the milk production trials.

Apart from misrepresentations, the industry claims are usually restrictedly based on small numbers of cows (seven to ten per test group), reflecting TAD efficacy protocols in which adverse veterinary effects were only incidentally noted. Claims that increased hormone levels are not found in milk are suspect since they do not reflect anticipated dose-response relationships and do not reflect increased plasma levels noted in several studies (see "Potential Adverse Public Health Effects").

The industry claims for the synthetic hormones are based on a complex of strategies. These exaggerate efficacy; omit reference to, trivialize, or dismiss documented adverse veterinary effects; and reflect misleading manipulation of data. Furthermore, these claims fail to reflect the absence of critical studies that could elicit further information on adverse veterinary effects and, even more critically, on adverse public health effects. The past success of the industry strategies also reflects suppression of data, on the alleged grounds of trade secrecy, and the unbalanced and indentured nature of in-house and academic research on synthetic milk hormones. Certainly, the documented evidence of adverse veterinary effects of milk hormones justifies the highest index of suspicion as to undocumented industry claims on human safety.

ADVERSE VETERINARY EFFECTS

Available data on adverse veterinary effects in cows hyperstimulated by daily injections of the synthetic hormones are sparse and are largely based on incidental findings in small-scale milk production trials, in the absence of multilactational and multigenerational toxicological studies. The significance of these findings, to which no reference is made in industry promotional literature, is emphasized by the small size of the trial groups, ranging from seven to 47 cows for each treatment group. The gross statistical insensitivity of such trials has recently been emphasized. "At least 2,423 cows would be needed in each group to detect an increase in disease frequency from 5 to 10 percent, and at least 11,773 cows in each group for a change from 1 to 2 percent" (4). The importance of stress-related diseases associated with prolonged elevation in plasma levels of BGH has been strikingly confirmed in transgenic pigs in which there were "significant improvements in both daily weight gain and feed efficiency." However, these pigs also developed "a high incidence of gastric ulcers, arthritis, cardiomegaly, dermatitis, and renal diseases" (5). It should be noted that these scientists, unlike their indentured dairy science counterparts, carefully investigated adverse veterinary effects as well as productivity.

Negative Energy Balance

Biosynthetic milk hormones induce a prolonged negative energy balance, similar to that in the rising phase of lactation, for at least eight weeks, during which increased milk production is paralleled by "reduced total body fat," excessive tissue loss, and hypertrophy of foregut tissue (6). This sustained negative energy balance appears to be associated with increased stress, susceptibility to infectious disease, and measurable changes in the composition of milk.

Increased Incidence of Infectious Diseases

In the Cyanamid-Pennsylvania met-BGH trial, mastitis developed in four of eight cows at 12.5 mg/day and in two of seven at 50 mg/day. High somatic cell counts were observed at all dosages in the Monsanto-Missouri trial, and at 25 mg/day in the Cyanamid-Missouri trial (4). Additionally, a high level of unspecified infectious disease was noted in one of nine trials. An increased incidence of unspecified (and unpublished) infectious disease has recently been confirmed (7).

Reduced Fertility

Evidence of reduced fertility has been noted incidentally in four of nine milk production trials (4, 8). Such evidence is further supported by evaluation of the results of 59 industry or industry-sponsored trials recently reported in two supplements of the *Journal of Dairy Science* (Volume 70, Supplement 1, 1987, and Volume 71, Supplement 1, 1988). Reproductive data were cited in six of these 59 trials—only two of which involved second lactations—all of which uniformly demonstrated significant adverse reproductive effects (9). The overall conception or pregnancy rates of controls in these six trials were 89 percent versus 59 percent in injected cows. More marked effects were noted in one study with pregnancy rates of 82 percent in controls versus 41 percent in high-dose-level cows, although conception rates were similar in all groups (10). In general, these adverse reproductive effects were ignored or trivialized. Illustratively, in one of the six trials it was claimed that "reproductive performance did not differ from contemporary herdmates," although conception rates in controls were 100 percent versus 50 percent in injected cows (11). Again, another study claimed that "health measurements were not consistently altered by sometribove" (met-BGH), although conception rates were 95 percent in controls versus 79 percent in injected cows (12).

In addition to the inhibition of conception rates noted in some of the six trials, one of these demonstrated reduction in pregnancy rates in the absence of effects on conception (10). As recently recognized, "BST [bovine somatotropin, i.e., BGH] may affect embryo survival. In one study, the conception rate of BST-treated cows was not influenced. However, there was evidence that BST-treated cows particularly those receiving high doses maintained fewer pregnancies" (13).

Thus, even from a narrowly focused economic perspective, and ignoring costs of other adverse veterinary effects, increased productivity from the use of synthetic milk hormones could be more than offset by economic losses due to reproductive impairment (9).

Heat Intolerance

Heat intolerance was noted at two dosage levels in one of nine trials (4). Such intolerance could pose particular problems for uses of biosynthetic hormones in tropical climates.

Changes in Nutritional Quality of Milk

Available data on the effects of hormones on the nutritional status and composition of milk, including protein subfractions, vitamins, and minerals, are minimal. However, it is clear that the hormones induce a wide range of measurable changes in milk composition. Increased fat yields and concentrations have been noted (14). Additionally, there is a statistically significant increase in long-chain fatty acids and decrease in short-chain fatty acids (15); this is associated with reduction in casein, in relation to both total and true protein, which is likely to decrease cheese yields. Such significant changes in the composition of milk in hormonally treated cattle are becoming increasingly recognized (e.g., 8).

Questionable Efficacy of Milk Hormones

The adverse veterinary effects so far noted are not necessarily offset by improved milk production. Contrary to promotional claims, the effects of synthetic hormones on milk production are highly variable and inconsistent. In nine met-BGH trials, outstanding responses were obtained in two herds and very poor responses in another two herds. "About one-third of all BST-treated herds would be predicted to fall between the consensus low limit of 10 percent more milk and my estimate of minus 1 percent based on the nine trials" (16). In spite of strident industry denials, burnout or lactational crash has been noted in hormone-treated cattle, particularly at high dose levels (7, 9), although no data have as yet been made available on its incidence.

Other Growth Hormones in Milk

Apart from unresolved questions on incremental levels of synthetic hormones in milk, somatomedins such as insulin-like growth factors (IGF-1), whose endogenous production is stimulated by milk hormones, have been detected in the milk of cows treated with synthetic hormones. Based on the very limited available data, the milk of treated cows appears to sustain high levels of IGF-1, similar to those found in untreated cows after the first week of lactation (17, 18). Additionally, the normal inverse relationship between endogenous growth hormone and blood insulin levels is disturbed following BGH treatment (19).

Misuse of Milk Hormones

Apart from concerns about overdosage of lactating cows, the off-label use of synthetic BGH as a growth-promoting hormone in calves and sheep has also been reported. Such misuses are all the more likely in view of the absence of practical and

sensitive methods for detecting and monitoring hormonal levels in milk and meat. Also, the documented record of extensive misuse of growth-promoting animal sex hormones does not inspire confidence that milk hormones will be handled any more responsibly.

Critical Data Gaps

It should be stressed that no information is available from large-scale multilactational and multigenerational dose-response tests with synthetic hormones on a wide range of veterinary and related concerns. These include: milk production efficacy; alterations in the detailed biochemical composition of milk, its nutritional quality, and its suitability for cheese production; alterations in reproduction and fertility; detailed studies on the growth and health of calves of injected cows; endocrinological effects; biochemical, endocrine, and metabolic evidence of stress; stress-induced susceptibility to and increased incidence of viral infections, including bovine leukemia; increased levels in milk of antibiotics necessitated by increased bovine infections; allergenicity and immunogenicity of hormonal milk; response of hormone-treated cattle to vaccines; mobilization in milk of fat-soluble carcinogens from depot fat by the sustained lipolytic action of milk hormones; and identification and measurement in milk and meat of synthetic hormone residues and of incremental levels of IGF-1 and other somatomedins.

POTENTIAL ADVERSE PUBLIC HEALTH EFFECTS

An editorial in a highly conservative British medical journal recently warned that before the use of BGH can be considered commercially, "one would need to be completely reassured that the appropriate tests have been carried out thoroughly and professionally and that there is not the slightest hazard to human health" (20). In fact, the use of milk hormones poses serious risks of adverse public health effects that have not been adequately considered (7, 8), in spite of continued unfounded but strident industry and industry contractee assurances of safety. Apart from a wide range of information gaps that negate such assurances, there are some highly suggestive contrary data.

Relationship of Biosynthetic to Natural Milk Hormones

Industry claims that synthetic BGH is "natural" are false. Both BGH and met-BGH are xenobiotics (8). Natural BGH consists of 191 amino acid residues in linear sequence. The Elanco BGH, however, has a series of eight additional amino acid residues, known as linker proteins, at one end of the molecule (21); the more potent met-BGH has an alien methionyl terminal residue. In addition to such chemical differences, synthetic BGH is synthesized on a bacterial rather than a mammalian ribosome and its bacterial links have not been clipped off, resulting in possibly different biological activities from natural BGH. The FDA has recently admitted that

biosynthetic milk hormones "are about 0.5 to 3 percent different in molecular structure" from the natural hormone (22).

Biological Activity of Milk Hormones

The industry initially claimed that BGH was "species-specific" to cattle, and thus could not possibly have any effects in humans. However, BGH is now known to be active in a wide range of species, including goats, pigs, sheep, mice, and even fish. Accordingly, the industry has changed its position and now claims that BGH is "species-limited" (23).

Natural BGH derived from pituitary glands was shown in the 1950s to have "no effect on human growth, sexual development or well-being" (24). Natural BGH is immunologically different from the human hormone and differs structurally in some 30 percent of its amino acid residues. While natural BGH is inactive in all primates, it should be noted that human growth hormone is only active in humans when given in high (milligram) doses. Additionally, some human dwarfs, Laron-type, are resistant to the treatment with human growth hormone unless it is administered together with androgens (25). Moreover, no studies on humans have been conducted with the synthetic hormones, especially the more potent met-BGH. Furthermore, it was demonstrated some 30 years ago that chymotrypsin digests of natural BGH are biologically active in humans, in whom they induce nitrogen retention (26); these considerations prompted unheeded recommendations to Monsanto some 26 years ago to undertake detailed studies on the biological activity of peptide fragments of synthetic milk hormones (7). Thus, the synthetic hormones could be biologically active in humans following absorption of novel peptides, formed during pasteurization or during proteolytic digestion in the alimentary canal. Also, the intact hormone molecule could be absorbed into the blood from the digestive tract, particularly in newborn infants prior to closure time and in infants or adults with impaired protein digestion in diseases such as cystic fibrosis; absorption of intact protein molecules has been demonstrated in newborn babies and some adults (7, 8). The industry recently admitted that "some proteins are absorbed into the blood stream without being fully digested" (24).

Industry claims that increased levels of synthetic hormones are not found in the milk of injected cows (3) using radioimmune assays. However, there are no available data on the comparative sensitivity and specificity of these assays for natural as opposed to synthetic hormones. Additionally, it is likely that administration of synthetic hormones will inhibit endogenous production of natural BGH and its levels in milk (25). In a recent publication purporting to confirm these claims, the upper range of levels in cows treated with 25 mg/day of synthetic BGH was more than 50 percent in excess of controls (27). Furthermore, dose-response relationships for plasma levels of synthetic BGH in the range of 5-30 ppb (ng/ml) have been reported (28). Up to 700 percent increased plasma levels have been reported following synthetic BGH dosing in late lactation (29); others have confirmed such elevations (e.g., 30). Paradoxically, excess levels of synthetic hormones have not been reported in milk assays by industry and its contractees. Clearly, the milk of treated cows should be assayed by independent scientists using techniques that have yielded clearcut results with plasma.

Biological Activity of Growth Factors

There is a growing consensus that the mechanism of action of the pituitary growth hormone is through the induction of somatomedin growth factors, particularly IGF-1 (31). From all criteria, bovine and human IGF-1 appear identical (31, 32). Most of the specific activities of natural BGH, including milk production, gluconeogenesis, diabetogenesis, nitrogen retention, lipolysis, mitogenesis, and adipose tissue and bone growth, are mediated through somatomedins. Moreover, mammary gland receptors for IGF-1 have been identified (33).

Increased IGF-1 levels have been reported in goat's milk following synthetic BGH treatment (17). As subsequently briefly reported, high levels of IGF-1 are found in normal cow's milk immediately after calving, falling to 1–5 ng/ml by 200 days (18). However, levels induced by daily injections of BGH were sustained at 6–20 ng/ml. Thus, irrespective of the possible activity in humans of synthetic BGH digestion products, mitogenic effects could be indirectly induced in humans by sustained incremental levels of IGF-1 and other somatomedins following absorption of their intact molecules or biologically active fragments from the gastrointestinal tract. Such effects could include premature growth stimulation in infants, gynecomastia in young children, and breast cancer in women.

A recent publication insisting that BGH technology is sound nevertheless warned that (31):

> Investigation of IGFs requires attention, particularly where animal health and food residues are concerned since they possess many biological activities and are immunologically and biologically similar among species. . . . Some concerns arise as to the possibility of abnormal levels of IGF-1 in the milk of BGH-treated cows and, with it, consumer health.

Another publication warns (18):

> The implications of IGF-1 in milk for the human infant cannot be determined until we know more about the activity and function of milk IGF-1 in the newborn. However, total growth factor activity in cow's milk, as assessed by a cell proliferation test *in vitro* which also detects components other than IGF-1, is not altered by BST treatment.

In addition to detailed studies on IGF-1 levels in the milk of BGH-treated cows, the effects in humans of increased levels should be studied with priority, particularly since some consumers have already and unknowingly been exposed to BGH milk; this population at risk should be identified and subjected to long-term surveillance. Systematic studies on IGFs should include dose-response in vitro investigations with human cells and tissues and dose-response studies in infant and adult primates, with a view to defining the effects of incremental milk levels in humans.

Activity of Hormonally Induced Stressor Metabolites

The levels in milk of stressor metabolites induced by synthetic hormones and somatomedins, such as epinephrines, catecholamines, and cortisol, should be

determined by sensitive and specific assays. The stressing action in humans of these metabolites should be investigated.

Infectivity of Hormonal Milk

The stressing effect in cows of synthetic hormones and somatomedins may induce immunosuppression and activate latent viruses, such as bovine leukosis virus (BLV) and bovine immunodeficiency virus (BIV), which may well increase susceptibility to other infectious agents. Levels of such viruses in hormonally treated milk and their human infectivity should be investigated with particular reference to risks of immunosuppression and leukemia. The relationship between these viruses and the AIDS (acquired immune deficiency syndrome) complex is of further concern, particularly in view of the high level of homogeneity between BIV and human immunodeficiency virus type I, and the infectivity of BLV to chimpanzees.

Antibiotics in Hormonal Milk

The increased incidence of infectious diseases, which has been noted in efficacy trials and which is presumably stress induced, is likely to result in increased antibiotic treatment and antibiotic levels in milk. Accordingly, the incidence of infectious diseases and of antibiotic levels in milk should be investigated with particular reference to the risks of induction of antibiotic resistance in the general population.

Allergenicity of Hormonal Milk

The allergenic and immunogenic effects in humans of met-BGH in milk, and of novel peptides resulting from its pasteurization or digestion, should be investigated. This is of particular concern in view of the substantial evidence on the high incidence of antibody development in humans treated with methionyl human growth hormone, rather than with the natural hormone (34).

Fat-Soluble Carcinogens in Hormonal Milk

The fat and milk of cattle are contaminated with a wide range of carcinogens, including pesticides such as heptachlor epoxide and dieldrin and xenobiotics such as polychlorinated biphenyls (PCBs) and tetrachlorodibenzodioxin. The lipolytic effect of hormonal treatment is likely to mobilize carcinogens from body fat and increase their milk levels, a matter of particular concern to young infants. For these reasons, possible incremental levels of fat-soluble carcinogens in hormonal milk should be determined.

Nutritional Quality of Hormonal Milk

The nutritional quality of hormonal milk should be investigated in multilactational and multigenerational tests. As recently emphasized, such data "on detailed components of milk, e.g., casein fractions, are not available" (27). Available data, however,

demonstrate major increases in long-chain saturated fatty acids relative to medium- and short-chain saturated fatty acids, and up to 27 percent higher fat levels in hormonal milk (14). Dose-response relationships between milk fat and synthetic BGH have also been reported (28).

Misuse of BGH and Met-BGH

In the event that registration should ever be granted to these biosynthetic hormones, there would be no practical method to prevent their extensive off-label misuse, as is well documented for sex growth hormones, or to detect and even monitor for such misuse. It is thus highly likely that these hormones would be administered at excessive dosages to lactating cows and as growth stimulants to calves, sheep, and other cattle, increasing still further the exposure of the general public to these highly potent biological agents.

PUBLIC POLICY RECOMMENDATIONS

1. The manufacture, domestic sale, and export, including foreign licensing agreements, of biosynthetic milk hormones should be banned immediately. This ban should remain effective until a wide range of concerns on public health and veterinary safety have been posed and fully resolved.
2. The sale of milk, milk products, and meat from hormone-treated cows should be embargoed immediately. To ensure compliance, industry and its academic contractees must be required to immediately identify all past and currently treated herds.
3. Attempts should be made to identify and place under long-term medical surveillance all consumers, especially infants, who are at potential risk from having consumed hormonally contaminated milk, milk products, and meat.
4. The industry and its academic contractees must be required to make immediate full disclosure of all unpublished data and reports; claims for confidentiality must be legally preempted on the grounds of overriding concerns about public health and welfare.
5. The conduct of industry and of its academic contractees with regard to suppression and manipulation of data should be subject to Congressional investigation.
6. The conduct of the FDA in granting an INAD exemption for the testing of synthetic hormones in cows and approving the sale of hormonal milk, in apparent violation of the 1968 FFDCA amendments, together with its unfounded assurances of safety, should be subject to legal challenge and Congressional investigation.
7. The industry must be required to develop and undertake multilactational and multigenerational dose-response and other protocols appropriate for the investigation of potential adverse public health effects from hormonally contaminated milk, milk products, and meat. Such research should be subject to ongoing independent review. These protocols must include: specific and sensitive assays for synthetic hormones and somatomedins; investigation of the biological activity of these hormones and growth factors in milk; analysis of milk for stressor chemicals; investigation of the biological activity of such stressor chemicals at levels expected in hormonal milk; analysis of milk for antibiotics necessitated by treatment of stress-induced infections

in lactating cows; analysis of milk for stress-induced or activated viral agents; analysis of milk for increased levels of fat-soluble carcinogens mobilized by synthetic hormones; investigation of the allergenicity and immunogenicity of synthetic hormones and of any derived novel peptides; investigation of the response to vaccines of treated cows; and detailed analysis of the nutritional quality of hormonal milk.

8. The industry must also be required to fund research in accordance with independently approved protocols, which should be awarded, supervised, and otherwise administered by a neutral, independent intermediary such as the National Institutes of Health or the National Science Foundation.

9. Pending action at the federal level, state legislatures should take immediate initiatives including labeling milk, dairy products, and meat from cows treated with synthetic BGH and banning the state sale of these products. State legislatures should also investigate the conduct of state universities in their contractual relations with industry, their involvement in the sale of unlabeled hormonal milk, and their misleading assurances of the safety of synthetic milk hormones.

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Note added in proof

The following effects in BGH-treated cows have received striking recent confirmation: increased incidence of infectious diseases (Otterby, D. E., et al. *J. Dairy Sci.* 72(Suppl. 1): 329, 1989); reduction in fertility (Morbeck, D. E., et al. *J. Dairy Sci.* 72(Suppl. 1): 345, 1989; five other reports in the same issue are further confirmatory); increased levels of IGF-1 in milk (Prosser, C. G., et al. *J. Dairy Res.* 56: 17–26, 1989). On August 23, 1989, the Foundation on Economic Trends, in association with farm, animal welfare, consumer, and environmental groups, petitioned FDA to ban sales of dairy products from BGH-treated cattle; the petition was based on a draft of this article. Simultaneously, national supermarkets banned dairy products from BGH-treated cows. The author's September 6 Wisconsin State testimony triggered a large-scale defensive reaction and public relations blitz by the industry. Illustrative is a Consumer Information Program by Elanco, Monsanto and Upjohn entitled, "You've had BST and Cookies All Your Life," which, apart from gross misrepresentations, falsely equates synthetic with natural BGH.

Direct reprint requests to:

Dr. Samuel S. Epstein
Health Resources Management (M/C 922)
School of Public Health West
University of Illinois at Chicago, Box 6998
Chicago, IL 60680

Section on Environmental Health Policy

QUESTIONS AND ANSWERS ON SYNTHETIC BOVINE GROWTH HORMONES

Samuel S. Epstein

Questions are posed and answered on synthetic bovine growth (milk) hormones (*s*-BGH), covering a wide range of areas of critical international concern. These areas include: the data base on *s*-BGH; efficacy and benefits to the dairy industry; veterinary effects; public health effects; Food and Drug Administration approval; and the FDA review process.

Natural bovine growth hormone (*n*-BGH) is a protein hormone that controls bovine growth and lactation. Synthetic bovine growth hormones (*s*-BGH) are manufactured by recombinant DNA biotechnology by the Agriculture Chemicals Division of Eli Lilly and Company (Elanco) in conjunction with the Dow Chemical Company, the Upjohn Company, American Cyanamid, and Monsanto.

Six years ago, the Food and Drug Administration approved the use of *s*-BGH in large-scale productivity trials, and the sale to the public of unlabeled milk and meat from these trials. The FDA has announced that it proposes to approve the commercial use of BGH in the near future. The industry expects national and international sales of approximately $500 million annually.

THE DATA BASE ON *s*-BGH

Question: What is the source of the available data base on *s*-BGH?

Answer: The data on which the FDA review and approval process is based have been generated and interpreted exclusively by industry and by its academic contractees and consultants in some 22 U.S. university dairy science departments, to the exclusion of any input by independent scientists. Additionally, no independent scientists have been directly or indirectly involved at any stage of the FDA review process. A detailed independent scientific review, with full supportive references, has recently documented substantive evidence of adverse veterinary effects, besides raising critical questions on public health hazards to consumers from consumption of dairy products and meat from animals treated with *s*-BGH (1).

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Q: Does the track record of the BGH industry justify confidence in the validity of its data base on toxic chemical products that the industry has attempted to market or has marketed in the past?

A: No. There is fully documented evidence that the data base of these industries and their indentured academics has been self-interested and highly unreliable, reflecting manipulation, suppression, distortion, and destruction of data on a wide range of products, including animal feed additives and drugs, pesticides, detergents, plastics, and other industrial chemicals (2–6). The track record of these industries is thus fully reflected in their misconduct in emerging fields of commercial biotechnology.

EFFICACY OF *s*-BGH AND BENEFITS TO THE DAIRY INDUSTRY

Q: Does the evidence support industry claims that administration of *s*-BGH increases milk production by 10 to 25 percent and that this will result in substantial benefits to dairy farmers?

A: No. Independent analysis demonstrates that increases in milk yields are highly inconsistent and variable (7–9). Taking into account the costs of *s*-BGH (estimated to be in the range of 25 to 75 cents per cow, per day) and extra feed—apart from the currently poorly recognized adverse veterinary effects, particularly reproductive—these data challenge the validity of industry claims on efficacy and benefits. Furthermore, available evidence indicates that increased milk production, contributing further to the national surplus and thus leading to a reduction in milk prices, is likely to result in a severe economic impact on the dairy farming industry, particularly on small dairy farms. Additionally, the reduction in casein levels noted in milk from *s*-BGH–treated cows may adversely affect the cheese industry.

VETERINARY EFFECTS OF *s*-BGH

Q: Does evidence support industry claims, endorsed by the FDA, that *s*-BGH administration is safe for cattle?

A: No. Industry claims of safety are based on data derived only incidentally from inherently insensitive productivity trials, based on small numbers of cows, as opposed to appropriate and statistically valid toxicological tests, including multi-lactational and multi-generational studies, based on larger numbers of animals. Nevertheless, available data from these trials clearly demonstrate a high incidence of adverse effects, particularly clinical or subclinical mastitis and impaired reproductive performance (1, 7–9); also reported are severe and persistent injection site reactions, and lameness in heifers and cows. However, with the acquiescence of the FDA, the BGH industry has discounted, trivialized, or misinterpreted its own data on such adverse effects. There are also informal reports on other adverse effects including burnout or "lactational crash," and deaths associated with fatty degeneration of the liver. In view of this substantive evidence on adverse veterinary effects, it is critical that Investigational New Animal Drug Application (INADA) industry data be made available for detailed review by the independent scientific community.

The increased incidence of infectious disease in cows hyperstimulated by daily injections of *s*-BGH, apart from other toxic effects such as heat intolerance, is highly suggestive of stress reactions. Nevertheless, there are no available data on the investigation of stress in *s*-BGH–treated cows, immune function, and the activation of latent viruses. The absence of such data is critical in view of recent evidence on serious and lethal stress diseases associated with elevated BGH levels in transgenic pigs (1). Also, there are no available data on a wide range of other metabolic endocrine and biochemical functions in *s*-BGH–treated cows.

PUBLIC HEALTH EFFECTS OF *s*-BGH

Q: Does evidence support promotional industry claims equating or implying the identity of *n*-BGH and *s*-BGH?

A: No. There are significant chemical and molecular differences between *s*-BGH and *n*-BGH. *s*-BGH contains up to eight additional amino acid groups at one end of the molecule; the FDA has recently admitted that there are some 3 percent structural differences between these hormones. It is well known that apparently minor structural variations—for example, involving only one or two amino acid groups in a protein molecule—can profoundly alter biological activity.[1] Additionally, there is no available information on the presence of nucleic acid and other bacterial contaminants in *s*-BGH.

Q: Can current industry analytical tests detect *s*-BGH in milk?

A: No. Industry has recently admitted that its current test procedures do not differentiate between *n*-BGH and *s*-BGH. Also, no information is available as to whether industry has yet developed sensitive tests capable of specifically differentiating between these hormones, and what, if any, the results of such tests are; there is a critical need for the disclosure of these data. In addition, *s*-BGH administration is likely to reduce normal (endogenous) production of *n*-BGH, so that most BGH in milk of treated cows is likely to be *s*-BGH rather than *n*-BGH.

Q: Does evidence support the industry and FDA claims that milk and meat from *s*-BGH–treated cows are safe from humans?

A: No. The evidence is based on the following: studies in the 1950s showing that administration of *n*-BGH to human dwarfs did not result in increased growth; claims that there are no increased BGH levels in milk from *s*-BGH–treated cows; and claims that any *s*-BGH consumed by humans would be digested and inactivated. The human dwarf data are irrelevant because they were based on tests with *n*-BGH and not *s*-BGH, apart from other considerations including the need to administer androgenic steroids together with human growth hormones to obtain any effects on growth in some clinical categories of dwarfs. Furthermore, proteolytic digests of

[1] For instance, sickle cell anemia is associated with the substitution of a single amino acid group (a glutamate is replaced by a valine) in a hemoglobin molecule; retinitis pigmentosa, an inherited disorder leading to blindness, is associated with the substitution of a single amino acid group in the rhodopsin or visual purple molecule.

n-BGH are metabolically active when injected in humans and induce metabolic effects in hypopituitary humans similar to those following administration of human growth hormone. Thus, peptide fragments of *s*-BGH, formed during pasteurization or in the human alimentary tract, could be absorbed and induce a wide range of potential adverse effects, particularly allergic and immunogenic; also, absorption of intact protein molecules is well recognized, particularly in infants. It should be emphasized that no data are available on gastrointestinal absorption of *s*-BGH and its peptide fragments in humans, or on the biological activity in humans of these synthetic molecules.

Similarly, there are no valid data on the detection and analysis of *s*-BGH in milk and meat products, although increases in plasma levels of up to 700 percent have been reported. Very high levels of *s*-BGH would also be expected in meat as a result of persistent injection site reactions. Concerns on the potential hazards of *s*-BGH in milk and meat are further heightened by the failure of the FDA to require a preslaughter withdrawal period, even though this was strongly recommended by its own scientists in 1982 and 1983.

Other potential public health concerns for which no data are available relate to the presence of potent and potentially toxic contaminants in milk and meat from *s*-BGH–treated cows. These include elevated levels of IGF-1, a species cross-reactive cell-stimulating growth factor; absorption of intact molecules or active peptide fragments of IGF-1 could possibly induce premature growth in infants and adverse cell-stimulating effects, such as promoting breast cancer. Also, abnormalities in the biological behavior of IGF-1 could be induced by *s*-BGH (10). Other possible contaminants include stressor chemicals; antibiotics used in the treatment of cattle with infections induced by *s*-BGH; and viruses, particularly leukemia/lymphoma and AIDS-like viruses, activated by *s*-BGH. Whether or not any of these potential concerns pose real public health hazards can only be determined by detailed long-term investigations by qualified and independent scientists.

Q: Did the FDA require the industry, in accordance with 21CFR 514.1, to submit full reports of adequate tests by all methods reasonably applicable to show whether or not *s*-BGH is safe for human use as suggested in the proposed labeling?

A: No. The extensive laboratory animal safety studies necessary to establish drug withdrawal times and human food safety of new drugs were not required.

FDA APPROVAL OF *s*-BGH

Q: What was the basis of the FDA decision, some six years ago, to allow large-scale investigational trials on *s*-BGH by industry and its academic contractees and to allow the sale to the uninformed public of unlabeled milk, milk products, and meat from unidentified herds?

A: The FDA action was largely based on its statement that humans are normally exposed to BGH in milk, falsely implying the identity of *n*-BGH and *s*-BGH, and its unsupported claim that *s*-BGH is not biologically active in humans.

Q: Has the FDA stated its position on the future commercial approval of *s*-BGH?

A: Yes. The FDA has stated that *s*-BGH will be commercially approved in the near future, prior to which the FDA will submit a summary of its scientific findings to a peer-reviewed journal. However, there is no indication as to whether such peer review will be conducted by independent scientists, as opposed to industry scientists or its academic contractees or consultants. [See note added in proof.]

Q: Has the FDA determined whether the conditions of use, recommended or prescribed in the proposed *s*-BGH label, are reasonably certain to be followed in practice?

A: No. Once *s*-BGH is approved, the FDA will lose control over its use with regard to dosage for dairy cattle and off-label use in dairy and meat animals.

Q: Does the proposed labeling for *s*-BGH include reference to indications, dosages, route, methods, frequency and duration of administration, and any adverse veterinary effects, apart from unresolved questions on human safety?

A: No. Adequate labeling cannot be written for the use of *s*-BGH under over-the-counter regulations. This labeling requires that adequate directions for use must be understandable to laypersons. The management, genetic, and other variables encountered in dairy farming cannot be adequately described on a label. Furthermore, no reference to human safety concerns has been proposed.

Q: Is the proposed labeling adequate to ensure the safe veterinary use of *s*-BGH?

A: No. The use of *s*-BGH would require monitoring of multiple variables to achieve increased production, particularly superior management; many small dairy farms cannot accommodate such requirements. If the directions are not followed, problems arising from use of the product could be attributed to misuse, for which neither the FDA nor industry would admit responsibility.

Q: Has the FDA proposed the labeling of *s*-BGH as a prescription drug in order to reduce consumer concerns on safety?

A: Yes. This, however, is not a valid or legal reason for labeling a production drug for prescription uses. Furthermore, a prescription drug would require a veterinary–client relationship that could not possibly be followed in large-scale commercial uses.

THE FDA REVIEW PROCESS

Q: Has the FDA Center for Veterinary Medicine (CVM) conducted the BGH review process in compliance with the Federal Food Drug and Cosmetic Act, the current regulations and requirements of 21 CFR 514.1, published guidelines, and unpublished policies, with regard to efficacy, veterinary safety, and human safety?

A: No. For details, see below (11, 12).

Q: Does the CVM have inappropriate contacts with the regulated industries, and is there evidence of inappropriate industry influence?

A: Yes. the CVM director has met regularly with personnel of the Animal Health Institute, a trade organization representing the regulated industries. There is, however, no evidence that the director met with consumer groups concerned with food safety. There are also allegations that donations to a national political party were requested of applicants for the CVM directorship, and that such a donation was ultimately paid by the regulated industry. Growing evidence indicates that corporate lobbyists "enjoy almost unlimited access" to CVM officials, and that the review process is characterized by illegal gratuities, favoritism, and rigging of assignments to "cooperative" staff members (13).

Q: Has the FDA undertaken unprecedented and inappropriate actions in support of an INADA for *s*-BGH?

A: Yes. High-ranking CVM and other senior agency personnel have spoken out in support of *s*-BGH. It is unprecedented for the FDA to publicly support or otherwise advertise an approved or unapproved animal drug.

Q: Is there a precedent in the CVM for the review of products exclusively in a single Division, without appropriate input from other Divisions and qualified CVM experts?

A: No. The Production Drugs Division (PDD) sequestered the BGH data and excluded any role for the Division of Toxicology and for the Biometrics Branch of the Division of Biometrics and Information. Additionally, the PDD denied experienced and board-certified CVM personnel, particularly veterinary pathologists and toxicologists, free access to BGH data.

Q: Are CVM statisticians inappropriately performing data entry and statistical reviews for industry?

A: Yes. The PDD has utilized two statisticians almost full-time for over three years to enter and analyze data for the regulated industry, apart from selecting animals for exclusion from the data base without proper clinical input.

Q: Is the Biometrics Group being utilized in the standard manner for the analysis of BGH data?

A: No. Normal procedures have been abandoned. The chief of Biometrics no longer has final sign-off authority on the work of his reviewers, who instead regularly and improperly report to the director of the PDD.

Q: Are PDD personnel qualified for the review of the *s*-BGH data?

A: No. PDD personnel have no previous experience with production drugs in dairy cattle. The PDD did not seek the counsel of qualified veterinary and animal science experts, and was thus inappropriately dependent on and influenced by the expertise of the regulated industry and its academic contractees.

Q: Did the CVM require the industry, in accordance with 21 CFR 514.1, to submit full reports by all reasonably applicable methods to show whether or not *s*-BGH is safe and effective as suggested in the proposed labeling?

A: No. Based on past inspections and records, there is evidence that full reports have not been made; that data and procedures were improperly handled; that adequate testing in adequate numbers of animals was not conducted; that the data were confounded by inappropriate use of concurrent therapy with approved and unapproved products; that data entries were made by unqualified or inappropriately supervised individuals; and that a fully qualified "uncooperative" staff scientist was fired after raising critical questions on the veterinary hazards of *s*-BGH (14). Furthermore, in spite of the reported INADA and other data on a wide range of adverse veterinary effects, the CVM has acquiesced in industry claims on the safety of *s*-BGH.

Q: Did the CVM follow the published guidelines and regulations requiring public comment in the development of protocols for the investigation of efficacy and safety of *s*-BGH?

A: No. Instead, the CVM developed a unique internal Technical Assistance Document with the admitted intent of avoiding the requirement for public comment.

Q: Did the CVM require that the Target Animal Safety tests on *s*-BGH be conducted under appropriate laboratory conditions?

A: No. Testing under dose confirmation studies or field conditions of use was allowed, contrary to 21 CFR 514.1. This allowed the use of diagnostic and therapeutic procedures that could mask adverse veterinary effects.

Q: What other BGH-related animal drugs, besides *s*-BGH intended for dairy cattle use, are now under review in the FDA?

A: These include: *s*-BGH for beef animals; *s*-BGH growth hormone releasing factor; anti-somatotropin antibody; and insulin growth factors.

Q: Were qualified CVM personnel involved in the review process on human food safety?

A: No. Such review appears to have been cursory in the extreme and to have been conducted by unqualified PDD personnel. Illustratively, current human safety evaluation is being conducted by an ex-PDD staffer, with no background or qualifications in toxicology, veterinary medicine, or public health, acting as an FDA consultant and residing in Nova Scotia.

Q: How many *s*-BGH trials have been undertaken, by each named industry, involving how many herds and how many cows; how much milk, meat, and dairy products from these trials have been sold to the public over the last six years; how many members of the public have consumed such foods; have any tests or studies been conducted on any such consumers; and are any tests, studies, or future surveillance planned for such consumers?

A: No information is available on any of these questions.

Q: Is the FDA review process on *s*-BGH consistent with its track record for other animal drugs and feed additives?

A: Yes. It demonstrates reckless irresponsibility and regulatory abdication. This was fully recognized in a recent Congressional report which concluded that "FDA has consistently disregarded its responsibility . . . repeatedly put what it perceives are interests of veterinarians and the livestock industry ahead of its legal obligation to protect consumers . . . jeopardizing the health and safety of consumers of meat, milk and poultry" (15). Confirmation of such regulatory abdication is provided by the FDA's admission, in a November 1988 consumer report, that "illegal use of veterinary drugs can be an even greater threat to the public health than the illegal use of human drugs." These concerns are still further emphasized by the results of recent investigations demonstrating that up to 38 percent of milk sampled nationally is contaminated by illegal residues of antibiotics and animal drugs, posing grave potential public health hazards, including antibiotic resistance, carcinogenicity, and allergic reactions (13). In this connection, without public notification, the CVM has recently tripled the allowable residues in milk of new antibiotics used for treatment of bovine mastitis, a common complication in *s*-BGH–treated cows.

ADDENDUM

Review of confidential INADA files submitted by Monsanto to the FDA has confirmed evidence on a wide range of adverse veterinary effects induced by *s*-BGH, besides public health concerns, as previously reported by the author [*International Journal of Health Services* 20(1): 73–84, 1990] but stridently denied by the FDA and industry and its academic consultants. These adverse effects include a major reduction in pregnancy rates; a high incidence of mastitis, necessitating extensive treatment with unapproved antibiotics and drugs; chronic toxic effects, evidenced by increased weight of body organs and disseminated pathological lesions; injection site reactions, sufficiently severe to cause carcass damage; and elevated milk and blood hormone levels. The suppression of such data has raised further serious public health concerns, and emphasized the need for independent review of all INADA files on *s*-BGH and for a high-level investigation of industry and FDA misconduct.

Accordingly, on May 8, 1990, Congressman John Conyers (D, Mich.), chairman of the House Committee on Government Operations, requested Inspector General Richard Kusserow of the Department of Health and Human Services to immediately investigate the FDA for "abdication of regulatory responsibility" with regard to its review of *s*-BGH used to artificially boost milk production. Congressman Conyers further charged that "Monsanto and the FDA have chosen to suppress and manipulate animal health test data . . . in efforts to approve commercial use of BGH." In a prompt reaction to these revelations, Senator Patrick Leahy (D, Vt.) pressured the FDA into accepting an independent review of industry data by the National Institutes of Health to evaluate consumer hazards from milk produced by *s*-BGH–treated cows. European reactions and concerns are not lagging far behind, as illustrated by the following International Resolution on BGH, unanimously approved at an international convention in Bonn, Germany, on May 15, 1990.

> International Resolution on Synthetic Bovine Growth Hormone
> We, the undersigned, as U.S. and European farmers, consumer and citizen groups, and independent scientists on the International Day of Milk, 15 May, 90, recognize that Bovine Growth Hormone (bGH) is just the first of the new animal husbandry

biotechnologies. In today's world, these technologies are more likely to serve the interests of rich and powerful industries rather than the needs of consumers for safe food; family farmers and rural communities for economic stability; and third world countries for agriculture self-sufficiency.

Biotechnology industries claim that bGH will increase production and reduce costs. However, bGH is more likely to increase costs to the farmer; to destroy the small traditional family farm; to seriously damage animal health and welfare; to contaminate milk, dairy products and meat and pose major potential public health hazards.

- We demand an immediate international ban on the manufacture of bGH.
- We demand an immediate international ban on the manufacture of bGH-related products, such as bGH Growth Hormone Releasing Factors, anti-Somatotropin antibody, and Insulin Growth Factors.
- We demand an immediate ban on the international transshipment of bGH and bGH-related products.
- We demand an immediate ban on the sale of milk, other dairy products and meat from cows and from other meat animals treated with bGH and bGH-related products.
- We demand the immediate identification of herds of cattle and other meat animals treated with bGH and bGH-related products together with independent assurance that no milk, dairy products or meat will be sold to the public, and that all such products will be destroyed under independent supervision.
- There is more than adequate evidence that bGH induces a wide range of serious adverse health effects in cattle, and that consumption of contaminated milk from bGH-treated cows poses serious potential public health dangers.
- There is unarguable evidence from confidential files submitted by Monsanto, a major manufacturer of bGH, to the US Food and Drug Administration (FDA) confirming the evidence of these veterinary and public health hazards. For example, in flagrant contradiction to assurances of the industry and the FDA, milk from bGH-treated cows is contaminated with high levels of the synthetic bGH- hormone.
- We commend the EEC [European Economic Community] for its moratorium on the use of bGH.
- We commend Raymond McSharry, the EEC Agriculture Commissioner for recently proposing a ban on bGH on grounds of consumer concerns.
- We commend the European Parliament for its proposed ban on bGH.
- We commend the US Congress for their concerns with relation to bGH. In particular, we commend the actions by the House Committee on Government Operations, and the Senate Agriculture Committee in directing the General Accounting Office to investigate charges of misconduct by the FDA with regard to their review of bGH. We also commend Congressman Conyers, Chairman of the House Committee on Government Operations, for his more recent request to the US Inspector General for an independent investigation of Monsanto and the FDA for their willful suppression of critical information on the veterinary and public health hazards of bGH.
- We commend the states of Wisconsin and Minnesota for their recent moratoria on the sale of bGH dairy products in their states.
- We urge that immediate funding be made available to independent consumer-, farmers-, environmental-, animal rights and other concerned groups in order to ensure effective implementation of our recommendations.
- We urge an immediate investigation of Monsanto and other bGH manufacturing industries for possible violation of civil and criminal laws, both nationally and internationally, with respect to their deliberate misrepresentation and suppression of information on the hazards of bGH.

Based on our experience with bGH, quite apart from a wide range of other consumer products, drugs and industrial chemicals, it is clear, that the EEC and each individual nation world wide must fully and independently evaluate the detailed and raw industry data before accepting possibly misleading and self-serving assurances of safety.

- Finally, we reaffirm the rights of individual nations and states to set food and environmental safety policies without outside interference, and opposition to any General Agreement on Tariffs and Trade (GATT) proposal which would circumvent these rights, especially in the areas of new biotechnologies and food safety.

Bonn, May 14, 1990. CAMPAIGN AGAINST BGH, representing 30 German organizations; EUROPEAN FARMERS COORDINATION, representing 10 European organisations; NATIONAL FAMILY FARM COALITION, representing 30 US Organizations; PROF: SAMUEL EPSTEIN MD, representing independent scientists and the Rachel Carson Council, Washington, D.C.

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Note added in proof. As of July 1990, an FDA manuscript was in press in *Science*. The chief editor of *Science* is considered by this author to be prejudiced and biased against environmental and consumer concerns (*Int. J. Health Serv.* 20: 349–352, 1990), besides being an enthusiastic proponent of the commercial applications of biotechnology. The key reviewer of the FDA manuscript is Dale Baumann of Cornell University, who has been Monsanto's major contractee and consultant on BGH for nearly a decade.

Direct reprint requests to:

Dr. Samuel S. Epstein
Health Resources Management (M/C 922)
School of Public Health (West)
University of Illinois at Chicago, Box 6998
Chicago, IL 60680

LITERATURE SUMMARY (1985-1999) ON THE HAZARDS OF rBGH MILK*

Samuel S. Epstein, M.D.

*Largely based on Epstein, S. S. The Politics of Cancer Revisited, Appendix XII, p. 618-627, East Ridge Press, Fremont Center, N.Y., USA.

A. ADVERSE VETERINARY EFFECTS

Eppard et al, Unpublished "Confidential" Monsanto Report, January 13, 1987

"Small, multifocal adhesions were scattered-- in 16 of 33 cows administered (CP115099-F (rBGH), while none were observed in the six control cows. The adhesions were associated with--chronic pleuritis, chronic pericarditis, hyperplasia of pericardial membranes, epicardial fibrosis and/or villus hyperplasia of visceral pleura". Leakage of this report prompted Cong. J. Conyers (D. Michigan), Chairman of The House Committee on Government Operations, to charge Monsanto and FDA with "abdication of regulatory responsibility (as they) have chosen to suppress and manipulate animal health data -- in efforts to approve commercial use of rBGH".

Monsanto, 1993

The Package Insert for Posilac (rBGH) lists over 20 toxic effects. These include mastitis, injection site reactions, bloat and other digestive disorders, retained placenta and other uterine disorders, enlarged hocks, foot disorders, and the need for medication for such toxic effects.

FDA Freedom of Information Summary for POSILAC, 1994

"The relative risk of a treated animal showing signs of clinical mastitis during the treatment period was about 1.79 times that of a control animal."

Kronfeld, J. Am. Vet. Med. Ass. 204, 116-130, 1994

In the Monsanto toxicity study (Eppard et al, 1987), "the frequency of renal, pulmonary, mammary gland and joint lesions are related linearly to rBGH use up to 5 times the approved dose."

Willeburg, J. Am. Vet. Med. Ass. 204, 538-541, 1994

"The result of introducing rBGH will be an increase in incidence of mastitis in the dairy cattle population--the health of dairy cows will be at risk, and doubts about the welfare aspect have--caused the European Commission to delay its decision."

B. MISREPRESENTATION OF ADVERSE VETERINARY EFFECTS By INDUSTRY And INDENTURED SCIENTISTS

1. ANIMAL WELFARE

Monsanto's rBGH-drug, Posilac, has over 20 toxic effects listed on its label. At least nine are painful and disabling diseases. Use of this drug is thus inhumane. (Willeburg, Livestock Production Science 36:55, 1993; Willeburg, J. Am. Vet. Assn., 205:538-541, 1994).

FDA's approval of Posilac was based partly on the assumption that Posilac-induced mastitis is manageable. However, no experimental basis for this hypothesis has ever been reported. Moreover, a peer-reviewed scientific publication concluded that current preventive medical methods would probably be ineffective (Kronfeld, J. Am. Vet. Med. Assn., 204:116-130, 1994). Statistical analysis of the FDA's mastitis data has further confirmed this conclusion (Kronfeld, Am. Coll. Vet. Int. Med., Forum 12:682-684, 1994).

2. **SCIENTIFIC MISREPRESENTATION**

Documents released in 1994 (Posilac Labeling: FDA Freedom of Information summary; White et al., J. Dairy Science, 77:2249-2260, 1994) disclosed previous false denials of adverse health effects of rBGH. Illustrative was a large-scale outbreak of mastitis in rBGH-treated cows at Cornell University. Four of 42 control cows, in contrast with 14 of 42 rBGH-treated cows, developed mastitis. This statistically significant observation was at first trivialized: "Health variables--were not affected by treatment"(Bauman et al. J. Diary Sci. 71:205, 1988), and then clearly misrepresented: "No adverse health effects were observed--animals were in good health throughout the study" (Bauman et al. J. Diary Sci. 72:642-651, 1989). These false denials of rBGH-induced mastitis have been repeated elsewhere.

3. **DISTORTION OF PUBLIC POLICY**

Dale E. Bauman is an endowed professor at Cornell University, and consultant to Monsanto Company and the U.S. Congress Office of Technology Assessment (OTA). He authored the biologic basis for the OTA's economic predictions for rBGH(1991) as follows: "Catastrophic effects such as...mastitis--have been postulated to occur. However, no such effects have been observed in any scientifically valid public health studies." (OTA, Special Report, F-470, 1991).

This industry consultant also had substantial input into a USDA economic study in 1987. Claiming no adverse effects, the study recommended approval of rBGH to help American farmers be competitive in a global market. Also, Bauman's allegation that there are no adverse effects of rBGH on animal welfare was accepted by the White House (1994).

Thus, U.S. public policy on rBGH has been misled by the indentured scientific literature. This mischaracterization or suppression of evidence on serious adverse health effects has misled Federal agencies, such as the USDA and OTA, and heavily pressured the FDA to approve rBGH.

C. MAJOR DIFFERENCES BETWEEN rBGH And NATURAL MILK (apart from IGF-1)

Bauman et al, J. Dairy Sci. 68:1352-1362, 1985

Monsanto's rBGH stimulated twice the increase in milk yield than an equal dose of rBGH.

USAN & the USP Dictionary of Drug Names, page 510, 1988

Sometribove is "methionyl growth hormone (ox)." This alternative name revealed that Monsanto's rBGH does not have a natural amino acid sequence, but instead, has an extra methionine at the 191- position, which reflects manufacture by genetically altered bacteria rather than by the cow.

Baer et al, J. Dairy Sci. 72:1424-1434, 1989

In milk from untreated and rBGH treated cows, "serum protein (.65, 71%) and lactose (4.7, 4.80%) were higher and casein as a percent of true protein (80.2, 78.8%) was lower with the somatotropin treatment. Proportions of short-chain (11.6, 10.5%) and medium-chain fatty acids (58.6, 56.0%) were reduced and long-chain fatty acids increased (26.9, 30.4%) for control and somatotropin milks, respectively."

Capuco et al, J. Endocrinol. 121:205-211, 1989

Mammary activity of an enzyme, thyroxine-5'-monodeiodinase, which converts the hormone thyroxine to a more active form tri-iodothyronine, is doubled by rBGH treatment. Both hormones are present in normal milk, and the increased enzyme activity suggests that more tri-iodothyronine will be present in rBGH milk. The effects of tri-iodothyronine in rBGH milk on the thyroid status of human consumers needs serious investigation.

Food and Drug Administration, G. B. Guest, Director for Veterinary Medicine, Letter to Senator W. P. Winkle, State Capitol, Madison, Wisc., May 9, 1989.

FDA admitted that rBGH is "about 0.5 to 3 percent different in molecular structure" from the natural hormone.

Epstein, International Journal Health Services, 20:73-84, 1990

..."it is clear that the hormones induce a wide range of measurable changes in milk composition. Increased fat yields and concentrations have been noted. Additionally, there is a statistically significant increase in long-chain fatty acids and decrease in short-chain fatty acids; this is associated with reduction in casein, in relation to both total and true protein, which is likely to decrease cheese yields."

Kronfeld, J. Am. Med. Assn. 265:1389, 1991

"Significant dose-response relationships indicate that the concentration of methionyl-rbST in milk of cows treated with methionyl-rbST is raised progressively above the zero concentration of methionyl-rbST in milk of untreated cows."

Kronfeld, Science, 251:256, 1991

Cited a 1987 Monsanto toxicology report to the FDA which listed 9 drugs used as therapy for illness and infertility in rBGH treated cows that are not approved by the FDA for lactating cows. The use of unapproved drugs is likely to escape detection in routine screening of milk for drug residues"--. Thus adverse effects of rbGHs on the cow's health and fertility could indirectly affect human health through secondary drugs entering milk." This proposal of indirect human health risks posed by the increased use of medication, especially unapproved antibiotics to control extra illness' induced by rBGH in cows, was subsequently endorsed by the U. S. General Accounting Office (1992) which regards the milk monitoring system as ineffective, but it was rejected by the FDA (1993), which regards the milk monitoring system as effective and which expects farmers to use all drugs legally.

Eppard et al, (Monsanto), J. Endocrinol. 132:47-59, 1992

rBGH is more potent than BGH in increasing milk yields of lactating cows.

Mepham, J. Royal Soc. Med. 85:736-739, 1992

"Milk fat concentrations increase and those of protein decline--there are reports of increases up to 27% in the concentration of long-chain fatty acids--mean values seem likely to change in directions detrimental to the nutritional quality of milk,--health risks to individual consumers--would thus depend on how much of the milk consumed was from cows treated with bST."

Harbour et al, Techniques in Protein Chemistry 111:487-495, 1992

This study demonstrated further deviations of rBGH from natural amino acid sequences, namely the

presence of N-epsilon-acetyl groups attached to lysine at various positions. Monsanto's rBGH is 191-methionyl-144-N-epsilon-acetyl-BGH (Violand et al. Protein Science 3:1089-1097, 1994); it is chemically different from any of the natural variants of BGH.

Toutain et al, J. Animal Sci. 71:1219-1225, 1993

Several dose-dependent pharmacokinetic parameters differ significantly between bacteria-made rBGH and cow-made BGH. rBGH thus differs pharmacologically from BGH.

Monsanto, 1993, Posilac Package Insert

The packing insert for Posilac (rBGH) states: "The use of Posilac is associated with increased frequency of use of medication in cows for mastitis and other health problems."

Erhard et al, J. Immunoassay 15:1-19, 1994

This study demonstrated that methionyl-rBGH is immunologically different from natural BGH; however a specific assay for rBGH has not yet been required by the FDA. This finding also raises the possibility of immune interactions between human growth hormone and rBGH.

Kessler Federal Register 59(28):6279-6280, 1994

The FDA stated that there is "no significant difference" between the milks of rBGH treated and untreated cows. However, numerous statistically significant differences have been reported in the composition of rBGH milk compared to controls.

Millstone et al, Nature 371-647-648, 1994

Milk from rBGH treated cows contains significantly more somatic cells (dispersed pus cells), which reflect the bacteria present in the mammary gland. High somatic cell counts are regarded as unwholesome, and both American and European authorities are striving to lower somatic cell counts in milk to make it more wholesome and to protect public health.

CONCLUSION

rBGH milk thus differs from natural milk nutritionally, pharmacologically, immunologically and hormonally. It is also contaminated by rBGH, which differs chemically from BGH, levels, by a thyroid hormone enzyme, and often by pus and antibiotics, besides by increased levels of IGF-1.

D. INCREASED IGF-1 LEVELS IN rBGH MILK

Prosser, Lancet 1:1201, 1988

IGF-1 levels in rBGH milk are increased up to 20 fold.

Juskevich & Geyer, (FDA), Science 249:875-884, 1990

Based on six unpublished industry studies, FDA admitted that IGF-1 levels in rBGH milk were consistently increased and that these increases were statistically significant. These levels were still further increased following pasteurization.

National Institutes of Health, Technology Assessment Conference Statement on Bovine Somatotropin, JAMA 265:1423-1425, 1991

IGF-1 levels in rBGH milk are increased up to 8.5 fold; levels are also increased in meat.

Joint FAO/WHO Expert Committee on Food Additives, Fortieth Report, Geneva. June 9-18, 1992
Cited six unpublished industries studies confirming increased IGF-1 levels in rBGH milk. These included one by Monsantoo (Schams et al, 1988) reporting a four-fold increase, and another (Miller et al. 1989), reporting a further 50% increase following pasteurization.

Mepham, Journal Royal Soc. Med. 85:736-739, 1992
Increased levels of IGF-1 in rBGH milk are probably underestimated because of flawed and analytical techniques. Also, the IGF-1 may be more potent than normal as it is not bound to milk proteins. Furthermore, some IGF-1 is likely to exist in modified (truncated) form; this is underestimated by four-fold in standard measurements and is ten times more potent than normal IGF-1. (This may result in a forty-fold underestimate of IGF-1 levels in rBGH milk.)

Mepham et al, The Lancet 2:197, 1994
In their 1993 European marketing application, Lilly admitted that IGF-1 levels in rBGH milk could be increased by more than ten-fold.

Mepham & Schofield, International Dairy Federation Nutrition Week, Paris, June 1995
"There seems to be no doubt that the concentration of IGF-1 in milk is increased by rBGH treatment, although the extent of increase appears variable."

Epstein, International Journal of Health Services 26(1):173-185, 1996
Details evidence of major increases of IGF-1 levels, besides its increased potency, in rBGH milk.

E. PUBLIC HEALTH HAZARDS FROM INCREASED IGF-1 LEVELS IN rBGH MILK (apart from cancer)

Prosser et al, J. Endocrinol. 112:65, 1987
"The implications of IGF-1 in milk for the human infant cannot be determined until we know more about the activity and function of milk IGF-1 in the newborn."

McBride et al, Res. Dev. Agricult. 5:1-21, 1988
"Investigation of IGFs requires attention, particularly where animal health and food residues are concerned since they possess many biological activities and are immunologically and biologically similar among species...Some concerns arise as to the possibility of abnormal levels of IGF-1 in the milk of BGH-treated cows and, with it, consumer health."

Epstein, Int. J. Health Serv. 20:73-84, 1990
Adverse effects of increased IGF-1 levels in rBGH milk "could include premature growth stimulation in infants."

Juskevich & Geyer, (FDA), Science 249:875-884, 1990
FDA reported summaries of still unpublished (1988 Monsanto toxicity tests) on IGF-1. Oral administration of IGF-1 to mature rats for only two weeks induced statistically significant evidence of growth promoting (mitogenic) effects even at the lowest doses tested. Nevertheless, FDA relies on these tests in its claim that IGF-1 is "orally inactive."

American Medical Association, Council on Scientific Affairs, Biotechnology and the American Agriculture Industry, JAMA 265:1429-1436, 1991

"Further studies will be required to determine whether the ingestion of higher than normal concentrations of bovine insulin-like growth factor is safe for children, adolescents and adults."

National Institutes of Health, Technology Assessment Conference on Bovine Somatotropin, JAMA 265:1423-1425, 1991

"Milk from rBST-treated cows contains higher concentrations of IGF-1. The importance of the increased amounts of IGF-1 in milk from rBST-treated cows is uncertain.

Mepham, Journal Royal. Soc. Med. 85:736-739, 1992

"It would be imprudent to assume that the increased concentration of IGF-1 in milk rBST treated cows presents no risks to human health." Based on conservative assumptions, infants drinking rBGH milk would be exposed to levels of IGF-1 substantially in excess of recommended safety margins derived from 1988 Monsanto oral toxicity tests published in summary form by FDA (Juskevich & Geyer) in 1990.

Lasmezas et al, Biochem. Biophys. Res. Comm., 196:1163-1169, 1993

ICF-1 induced a dose-dependent increased expression of the protein prion gene (PrP) in cultured rat neuroblastoma (PC12) cells. PrP is "A housekeeping gene which is responsible for susceptibility to transmissible spongiform encephalopathies". This study raises unresolved questions on the possible effects of increased IGF-1 levels on susceptibility to bovine (BSE) and human prion disease (CJD).

Mepham et al, The Lancet 2:197, 1994

"We believe that the safety of rBST-milk has not been established with adequate scientific rigor because of possibly adverse effects of substantially increased concentrations of Insulin-like Growth Factor-1(IGF-1) in the milk of rBST treated cows.

Graefe zu Baringdorf, Friederich-Wilhelm, Letter to FDA Commissioner David Kessler, December 7, 1994

"We feel fairly confident in being able to demonstrate that the safety of European citizens who consume rBST products cannot be guaranteed. More and more scientific evidence, such as the recent pieces in the British medical journal The Lancet, is accumulating to support this position."

Geier et al, Cancer Invest. 13:480-486, 1995

The authors reported that IGF-1 specifically inhibited the lethal effects of different anti-cancer drugs on cultured human breast cancer cells. This suggests that IGF-1 is involved in the development of drug resistance "a major obstacle to the ultimate success of cancer therapy."

Resnicoff et al, Cancer Res. 55:2463-2469, 1995

The authors reported that IGF-1, interacting with its receptor, is highly protective against programmed cell death (apoptosis) of human and cancer cells in biodiffusion chambers in vivo. The practical implication of these findings was stressed. "The rate of cell death is an important determinant of tumor growth, and the extent of apoptosis could have a profound effect on the aggressiveness of a tumor." Anti-apoptotic effects could thus stimulate the growth and invasiveness of latent or cancers.

Mepham & Schofield, International Dairy Federation Nutrition Week, Paris. June 1995

"It is recommended that the safety of milk and milk products from cows treated with BST be reexamined in the light of recent reports which suggest that insulin-like growth factor-1 (IGF-1) in such milk is both bioactive in intestinal tissues and protected from degradation by casein in milk.--It is a matter of concern that were BST use to result in widespread milk avoidance, there might be significant adverse effects on public health."

Xian et al, J. Endocrinol., 146:215-225, 1995

Casein, the major milk protein, is highly effective in protecting IGF-1 from intestinal digestion, and preserving receptor binding activity in stomach and duodenal fluid in the presence of casein. (In striking contrast, salivary IGF-1 could be rapidly digested).

Epstein, International Journal Health Services. 26:173-185, 1996

Based on conservative estimates, an infant consuming rBGH milk would be exposed to IGF-1 levels over 100-fold in excess of standard safety margins, which would double its normal blood level over the course of one day. Furthermore, the extra IGF-1 could be up to 40-times more potent.

Schofield & Mepham, International Dairy Federation Conference, Johannesburg, South Africa, October 23, 1996

"It is now clear that the main action of the IGFs in transformation is through the inhibition of apoptosis induced by primary oncogenic mutations --".

Hansen et al, Consumers Union Report to the FAO/WHO Joint Expert Committee on Food Additives, September 1987.

Summarizes evidence on public health hazards of excess IGF-1 levels in rBGH milk with regard to: excess antibiotic levels in milk and antibiotic resistance; colon, breast, pediatric and other cancers ; and potentially increased risk of human prion disease (CJD).

F. rBGH MILK IS A BREAST CANCER RISK FACTOR

Furlanetto & DiCarlo, Cancer Res. 44:2122-2128, 1984

IGF-1 induces highly potent stimulatory (mitogenic) effects in cultured human breast cells. Furthermore, IGF-1 binds to specific surface receptors of these cells.

Pines et al, Gastroenterol., 80:266-269, 1985

An "enhanced risk" of breast cancer (SIR=3.5), besides a statistically significant increase in gastrointestinal cancers, was reported among a small group of acromegalics.

Glimm et al, J. Dairy Sci. 71:2923-2935, 1988

Administration of rBGH to cows, results in increased blood levels of IGF-1, and it's uptake and heavy concentration in mammary epithelial cells.

Reynolds et al, Gynecol. Oncol. 38:396-406, 1990

IGF-1 plasma concentrations are higher in breast cancer patients than in healthy controls. "Even if

there is no direct evidence that elevated plasma levels of IGF-1 reflect elevated levels of growth factor at the tumor level, the possibility exists that increased levels of circulating IGF-1 may contribute to breast tumor growth."

Lipman, J., National, Inst. Health Res. 3:59-62, 1991

IGF-1 and related growth factors are critically involved in the development of breast cancer and maintaining its invasiveness.

Rosen et al, Breast Cancer Res. Treat. 18(Suppl.):555-562, 1991

IGF-1 is a potent regulator of cultured human breast cancer cells.

Harris et al, New Engl. J. Med., 7:473-480, 1992

"It now appears highly likely that a series of growth factors are responsible, at least in part, for the evolution of normal breast epithelia to breast cancer, and that breast cancer cells maintain their malignant phenotype as a result of the effects of these growth factors. These factors include the insulin -like growth factor."

Pollak et al, Breast Cancer Res. Treat. 22:91-100, 1992

IGF-1 is more mitogenic to breast cells than the highly potent and carcinogenic estradiol. (While distinct from carcinogenesis, mitogenesis is likely to promote malignant transformation induced by estradiol.)

Lippman, Science 259:631-632, 1993

"A number of proteins have been shown to participate in aberrant growth of breast cancer cells. These proteins include several families of cell surface growth factor receptors (including) the IGF-1 family."

Pappa et al, Cancer Res. 53:3736-3740, 1993

"...plasma IGF-1 concentrations are higher in primary breast cancer patients,--the possibility exists that increased levels of circulating IGF-1 may contribute to breast tumor growth." Furthermore, levels of IGF-1 breast cell receptors are some ten-fold higher in cancer than normal cells.

LeROITH, D., Ann. Int. Med. 122:54-59, 1995

In a summary of information presented at a 2/23/94 NIH Conference on IGF-1, it was concluded: "IGF's are important mitogens in many types of malignancies.--IGFs are likely to be involved in breast cancer at the level of tumor growth and perhaps at the level of initial development and later metastases."

Epstein, International Journal of Health Services 26(1):173-185, 1996

Documents a wide range of converging lines of evidence strongly incriminating excess IGF-1 levels in rBGH milk as a risk factor for breast cancer.

Orme et al, J. Endocrinol. 148(Suppl.):OC22, June 1996

Based on a retrospective study of some 1400 acromegalics in 15 U.K. centers, a statistically significant excess of breast cancer mortality, and also of colon and overall cancer mortality, was reported.

Schofield & Mepham, International Dairy Federation Conference, Johannesburg, South Africa, October 23, 1996

"High levels of circulating IGF seem to be predisposing for the generation of breast cancer, but it is unclear whether this reflects a direct effect--". However, contrary to explicit evidence on the systemic effects following oral administration of IGF-1 to adult rats (Juskevich & Geyer, 1990), the authors stated that: "Quantitative considerations based on the uptake of IGF-1 from the gut into the circulation also indicate minimal risk". Furthermore, the authors appear surprisingly unaware of epidemiological evidence on the increased incidence of breast cancer in acromegalics.

NG et al, Nature Medicine 3:1141-1144, 1997

Dosing aged monkeys with IGF-1, over a broad range of concentrations extending down to the physiological, induced a highly significant increase in breast size and potent mitogenic effects on mammary epithelia. The authors warned of risks of breast cancer from treating post-menopausal women with IGF-1 or growth hormone, which acts by increasing IGF-1 levels, to delay the effects of aging.

Hankinson et al, The Lancet 351:1393-1396, 1998

In a prospective study of 300 healthy nurses, those with elevated IGF-1 blood levels, about 10% in excess of controls, were shown to be strongly associated with up to a 7-fold subsequent risk of premenopausal breast cancer. This risk factor appears greater than most others with the exception of a strong family history.

G. rBGH MILK IS A COLON CANCER RISK FACTOR

Pines et al, Gastroenterol. 80:266-269, 1985

A statistically significant increased incidence of gastrointestinal cancers was reported among a group of 48 acromegalics. Additionally, an "enhanced risk" of breast cancer was observed (SIR=3.5).

National Institutes of Health. Technology Assessment Conference Statement on Bovine Somatotropin. JAMA 265:1423-1425, 1991

"Whether the additional amount of IGF-1 from (rBGH) cows has a local effect in the esophagus, stomach or intestines is unknown."

Olanrewaju et al, Am. J. Physiol. 263:E282-E286, 1992

Infusion of IGF-1 into the intestine of rats at concentrations equivalent to those found in rBGH milk markedly increased the cellularity of mucosal cells.

Lamm, et al, Brit. J. Cancer 65:41-42, 1992

IGF-1 was shown to have potent mitogenic effects on 5 of 8 human colon cancer cell lines.

Sleisenger & Fordtran, eds. Gastrointestinal Disease, p. 1412, W. B. Saunders, Philadelphia, 1993

"Patients with acromegaly seem to have an increased tendency to develop colon cancers and adenomas. Although these studies inherently involve few subjects, consistently high prevalence rates of 6.3 to 25 percent for colon cancer and 14 to 35 per cent for adenomatous polyps were observed in acromegalics."

Burton et al, Can. J. Animal Sci. 74:167-201, 1994

Based on evidence including the presence of specific IGF-1 receptors in intestinal epithelial cells and the stimulation of enzymes of these cells by IGF-1 at levels 1/1000 below those claimed inactive by the FDA, the authors concluded: "It could be considered an oversight (for the FDA) to suggest that ingested IGF-1 is inactive.--Many more potential effects of ingested IGF-1 on the gastrointestinal tract and the local immune system of the gut need to be explored."

Challacombe & Wheeler, The Lancet 344:815-816, 1994

"The combination of IGF-1 in BST milk and IGF-1 normally excreted into the human gastrointestinal lumen would augment--concentrations of this hormone, increasing the possibility of local mitogenic effects on gut tissues."

Chaurasia et al, Regul. Pept. 50:113-119, 1994

"Since the growth factor is not protein-bound, its concentration in gut lumen may be high enough to exert biological activity."

Donovan & Odle, Annual Review Nutrition 14:147-167, 1994

"Studies suggest that orally administered IGF-1at least partially survives digestion, binds to the GI tract--and may stimulate cell proliferation. In addition, IGF-1 can be absorbed into the blood, where it may effect the secretion of other hormones."

Juul et al, Clin. Endocrinol 41:85-93, 1994

Blood levels of IGF-1 are significantly elevated in patients with gigantism (acromegaly) due to anterior pituitary tumors or hyperplasia.

Tremble & McGregor, In Treating Acromegaly, ed. Wass, pp. 5-12, Journal of Endocrinology Ltd., Bristol, England, 1994.

Increased rates of pre-cancerous polyps and colon cancer have been reported in acromegalics in whom levels of IGF-1 are significantly elevated.

Epstein, S. S., International Journal Health Services 26(1):173-185, 1996

Documents a wide range of converging lines of evidence strongly incriminating excess IGF-1 levels in rBGH milk as a risk factor for colon cancer.

Orme et al, J. Endocrinol. 148 (Supp.):OC22, June 1996

Based on a large retrospective study of some 1400 acromegalics in 15 U.K. centers, a statistically significant excess incidence and mortality of colon cancer, and also of overall and breast cancer mortality, was reported.

Wheeler & Challacombe, Gut. In Press 1996

IGF-1, and to a lesser extent Human Growth Hormone (HGH) and insulin, "alone or in combination" are involved in the regulation of crypt cell proliferation in the human intestine in vitro and possibly also in vivo .

Schofield & Mepham, International Dairy Federation Conference, Johannesburg, South Africa, October 23, 1996

"The effects of IGF-1 on gut proliferation and the acute sensitivity of the gut to IGF suggest that we should be most concerned about the generation of hyperplastic states in the gut, polyps, or ultimately, adenocarcinoma."

Hansen et al, Consumers Union Report to the FAO/WHO Joint Expert Committee On Food Additives, September 1997

Confirms and extends evidence detailed by Epstein, 1996 that excess IGF-1 levels in rBGH milk are a risk factor for colon cancer.

H. rBGH Milk Is A Prostate Cancer Risk Factor

Epstein, S. S., P. R. Newswire, March 16, 1998

As reported in a January 23, 1998 article in Science, men with high blood levels of IGF-1 (>270 ng/ml), are over four times more likely to develop full-blown prostate cancer than men with lower levels (<250 ng/ml). The report emphasized that high IGF-1 levels are the strongest known risk factor for prostate cancer, exceeding that of a family history, and that reducing IGF-1 levels is likely to prevent prostate cancer. It was further noted that IGF-1 stimulates the growth of normal and cancerous prostate cells, and that it blocks apoptosis of cancer cells thus stimulating the growth and invasiveness of prostate cancer.

Wolk et al, J. Nat. Cancer Inst. 90:911-915, 1998

A study on 210 men under the age of 70 with newly diagnosed prostate cancer revealed statistically significant excess blood levels of IGF-1 compared to matched controls. The authors concluded that: "IGF-1 likely plays an important role in the etiology of the disease."

Ma et al, J. Nat. Cancer Inst. 91:620-625, 1999

"In a prospective case-control study, 193 men with recently diagnosed prostate cancer were found to have a statistically significant excess of IGF-1 blood levels compared to controls matched for age, smoking, body mass, alcohol consumption, and other co-variants. The authors warned against the risks of administration of human growth hormone or IGF-1 for anti-aging purposes.

EUROPEAN COMMISSION
DIRECTORATE-GENERAL XXIV
CONSUMER POLICY AND CONSUMER HEALTH PROTECTION
Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

XXIV/B3/AW/R08/1998

Report on
Animal Welfare Aspects
of the
Use of Bovine Somatotrophin

Report of the
Scientific Committee on Animal Health and Animal Welfare

Adopted 10 March 1999

REPORT ON ANIMAL WELFARE ASPECTS OF THE USE OF BOVINE SOMATOTROPHIN

CHAPTER 1. INTRODUCTION:

1.1 Request for an Opinion

1.1.1 Mandate

The Scientific Committee on Animal Health and Animal Welfare is asked to examine the use of bovine somatotrophin (BST).

In particular, the Committee is invited to assess the effects and risks of using BST under normal conditions including the following aspects:

- the incidence of mastitis and other disorders in dairy cows;

- other aspects of the welfare of dairy cows.

In a parallel exercise, the Scientific Committee on Veterinary Measures related to Public Health is asked to report on possible direct and indirect adverse effects on consumer health caused by the use of BST.

1.1.2. Background

Council Decision 94/936/EC of 20 December 1994 amending decision 90/218/EEC concerning the placing on the market and administration of bovine somatotrophin (BST) prohibited the marketing and the use of BST in the EU until 31 December 1999.

The Council asked the Commission to entrust a Working Party of independent scientists with the task of assessing the effects of using BST, in particular as regards the impact of the use of this product on the incidence of mastitis. In this request, it is stated that "BST is an issue which gives rise to considerable interest among consumer, agricultural and industry interests. In this context concerns have been expressed about the safety to humans, animals and the

environment, the quality of milk, the economic and social consequences in agriculture, the climate for research and development, industrial competitiveness and trade implications".

The production of this report is therefore one of the steps requested by the Council prior to the review of the prohibition on the use of BST which should take place before 31 December 1999.

1.1.3 Previous Opinion

The Animal Welfare Section of the Scientific Veterinary Committee examined the general question of the use of substances administered to animals for non-therapeutic and non-prophylactic purposes in 1991. As a result it adopted the following statement;

"STATEMENT (1991) BY THE SCIENTIFIC VETERINARY COMMITTEE ON THE USE OF SUBSTANCES ADMINISTERED TO ANIMALS FOR NON-THERAPEUTIC AND NON-PROPHYLACTIC PURPOSES.

The Committee is concerned that in discussion about the use of products resulting from biotechnology procedures, such as recombinant bovine somatotrophin, insufficient attention is paid to effects on the welfare of animals treated with the product. Such a new product should not be licensed for general use unless adequate information from scientific studies of the welfare of animals treated with the product has been obtained and considered. Such studies should include measurements of welfare such as those of disease incidence, physical disorders, injuries, behaviour and physiology. These studies should be carried out over a period of the animal's life at least as long as the longest time that such an animal would be kept on a farm and in a variety of management conditions. Studies in commercial farm conditions should be included.

No comprehensive studies of the welfare of animals treated with recombinant bovine somatotrophin have been reported. Work on the effects on the incidence of mastitis and other production-related diseases indicates that some welfare problems may exist but more comprehensive studies are desirable to clarify the extent of the problems."

1.2 *Outline of Report*

The subject of chapter 2 of this report is a brief account of animal welfare and its scientific assessment. Chapters 3 to 5 review the biology of high yielding dairy cows, the usage of BST and the biology of BST action in cows. Chapters 6-12 provide and discuss data on the effects of BST on animal welfare. Conclusions and recommendations of the report are presented in chapter 13 and, finally, references are listed.

In this report the abbreviation BST is generally used to indicate recombinant bovine somatotrophin[1].

[1] Tropic factors affect direction or extent of body movement while trophic factors affect growth so

CHAPTER 2. Welfare Concepts and Assessment in relation to BST

2.1 The concepts of animal welfare

There is widespread belief that people have moral obligations to the animals with which they interact, such that poor welfare should be minimised and very poor welfare avoided. This has led to animal welfare being on the political agenda of European countries. In addition to political debate, the amount of information based on the scientific study of animal welfare has increased. Scientists have added to knowledge of the physiological and behavioural responses of animals and philosophers have developed ethical views on animal welfare. All agree that decisions about animal welfare should be based on good scientific evidence (Duncan, 1981, Ödberg, 1996; Simonsen, 1996).

The fact that farm animals are reared for commercial purposes should not cause us to forget that they are living and sensitive creatures which need to regulate their lives and avoid suffering. The concept of welfare has to be defined in such a way that it can be scientifically assessed and the term can be used in legislation and in discussion amongst animal users and the public. Welfare is clearly a characteristic of an individual animal and is concerned with the effects of all aspects of its environment on the individual. Broom (1986) defines it as follows: "the welfare of an animal is its state as regards its attempts to cope with its environment." Welfare therefore includes the extent of: success in coping, failure to cope which may lead to disease, injury and death, ease of coping or difficulty in coping and the associated pleasurable mental states and unpleasant states such as fear and frustration (Dantzer et al., 1983; Broom, 1988). Good welfare can occur providing the individual is able to adapt to or cope with the constraints it is exposed to. Hence welfare varies from very poor to very good and can be scientifically assessed (Broom, 1996, 1998, Broom and Johnson 1993). The word stress is used when there is failure to cope.

The welfare of a farm animal can be considered in relation to the housing and management conditions to which it is submitted. Welfare is good when all of needs associated with the maintenance of good health and needs to show certain behaviours to be met. Health is an important part of welfare and behaviour is important in many regulatory systems.

How this concept applies to animals which are submitted to an exogenous hormonal treatment aimed at increasing their productivity and having no direct benefit for the individuals to which it is administered is considered in the next sections.

2.2 The assessment of farm animal welfare

Farm animal welfare is assessed by a combination of indicators of its physical and mental components (Smidt, 1983). The scientific methods that are available for selecting these indicators, and establishing and interpreting scores, are detailed in several reviews (Moberg, 1985; Wiepkema and van Adrichem 1987; Broom, 1993; Broom and Johnson, 1993). In general, minimum early mortality, low morbidity, little or no risk of injury, good body condition, the ability to express species-specific activities including social interactions, exploration and play, and the lack of abnormal behaviour and of physiological signs of stress, including alterations of immune responses, indicate that there is no major animal welfare problem.

2.3 The assessment of the potential impact of BST on animal welfare

Any exogenous treatment that modifies the physiology of an organism with the objective of increasing its productivity is likely to impair welfare if the individual is not able to adapt to the physiological and metabolic changes this treatment induces. In addition, the treatment can impact on welfare indirectly, via its effects on body structure and function and factors that regulate behaviour at the sensory, perceptual, motivational and motor levels. The treatment under consideration could also increase mortality and morbidity risks, for example because of failure of basic regulatory physiological functions or the physiological function targeted by the treatment. All these possibilities need to be taken into account when assessing the possible effects on welfare of a new treatment.

If the treatment is administered by injection, it is important to verify that the injected product does not cause much pain or discomfort at the site of injection during or after the injection procedure.

In the case of BST, the following points must be considered for a proper assessment of the effects of this treatment on animal welfare:

(i) Injection site: Injected materials may cause localised or wide ranging painful effects. Comparative studies should involve normal test injections and placebo injections or no injection. Behavioural and physiological responses should be measured with and without human manipulation of the injection site area.

(ii) Mortality and morbidity: Early mortality or culling because of disease, injury or physiological system failure shows that the welfare has been poor. Hence the mortality rate on farm and the rate of culling for all but human error reasons are welfare indicators. In addition, welfare is poor if the incidence of production related diseases is higher in treated animals than in placebo-treated animals. If some weakness or abnormality means that the individual would be more likely to succumb to pathogen challenge, respiratory failure, poison accumulation, injury, etc. then the welfare is poorer than in an animal which does not have this weakness or abnormality. In a group of animals, such as a flock, house, herd or any other population unit, the amount of poor welfare caused by disease is a function of its incidence, severity and duration, as described by Willeberg (1991). Health indicators of animal welfare must also be studied with a broad population perspective. If the metabolic condition created by a treatment were responsible for an increased use of preventive or therapeutic veterinary medicines, the welfare would be poorer. Animals which may have leg pain or other pain should be compared with unaffected controls or the same individual after analgesic application or disappearance of all clinical signs.

(iii) Body condition and Reproduction: Welfare is poorer if body condition score is too low or if, at the other extreme, there is unbalanced organ function or damaging muscule hypertrophy. Reproduction is given high priority in the allocation of resources within an animal so, if given adequate fertilisation opportunities, individuals which are not already involved in reproductive processes are less likely to conceive or less likely to carry young to term, poor welfare is indicated.

(iv) Behaviour: Animals use behaviour as one of the important means of adapting to their physical and social environment. If such adaption is prevented, welfare will be poor. Various

behaviours including abnormalities of behaviour are indicators of pain, fear or other poor welfare. Some behaviours are indicators of good welfare.

(v) Physiology: Physiological indicators of metabolic stress or disturbance of the main regulatory functions, such as heart rate and adrenal hormones and signs of malfunction of the immune system are all indicators of poor welfare. Some physiological changes in brain and body may indicate good welfare. BST treatment should not create a state of metabolic stress nor interfere with the main physiological regulatory functions.

For an adequate assessment of welfare a wide range of indicators must be used, although single indicators can show that welfare is poor.

2.4 Conclusion

Animal welfare can be assessed in a scientific way and indicators of welfare include those of physiological states, behaviour and health. A proper assessment of the effects of BST on the welfare of dairy cows must be based on the whole range of indicators that are available to measure welfare in these animals.

CHAPTER 3 WELFARE PROBLEMS IN HIGH YIELDING DAIRY COWS

3.1 Biological functions which are modified when milk yield is high

The biology of dairy cows in relation to the high levels of milk production required from them in the modern dairy herd has been described in a variety of text books (e.g. Webster 1993). The cow is well adapted to eat fibrous plants whose energy and protein content are not high, for example grasses. The pasture plants preferred by modern cattle are those which are long enough for comfortable grasping with the tongue, are composed more of leaf than of stalk and contain an adequate proportion of water, fibre, protein and utilisable energy (Stobbs 1974, Fraser and Broom 1990, p.90).

If insufficient energy or protein are ingested by a lactating cow, which is the case at the beginning of lactation, she will utilise her body reserves (mainly adipose tissue) and, subsequently, body tissues such as muscle in order to continue lactation. If too much concentrate feed is given to a lactating cow, the accumulation of metabolites such as volatile fatty acids leads to a greatly increased risk of digestive problems and metabolic disorders. These may occur at the same time that a high milk yield is being produced so a high yield does not indicate the absence of problems. As Webster (loc. cit.) explains, ruminal overload and unstable fermentation can lead to acidosis and laminitis, whilst increased tissue mobilisation leads to, on the one hand weight loss and anoestrus and, on the other hand ketosis, which like acidosis, can result in fatty liver. Other clinical disease conditions are also more likely when digestive disorders occur. Disorders associated with an inappropriate dietary balance and prolonged high levels of milk secretion are mediated via a wide range of physiological changes in the cow.

3.2 Welfare problems in dairy cows

The major welfare problems in dairy cows are mastitis, foot and leg problems, conditions which lead to impaired reproduction, inability to show normal behaviour, emergency physiological responses or injury.

For a recent review of lameness, including the extent to which it is a welfare problem, see Greenough and Weaver (1996). Almost all animals which walk with a limp, or reduce walking to a low level, or avoid walking whenever possible suffer from some leg or foot pain. In some cases, walking is reduced because of pain in all four feet but the animal may not limp. The ability of cows with foot and leg problems to carry out various preferred behaviours is generally impaired and there may be adverse consequences for various other aspects of their normal biological functioning. Clinical disorders of feet and legs in dairy cows always mean some degree of poor welfare and sometimes means that there is very poor welfare indeed. Measurements of the extent to which some degree of lameness occurs in dairy cows include 35 - 56 cases per 100 cows per annum in the USA, 59.5 cases per 100 cows per annum in the UK and more than 83% prevalence in cows kept in loose housing systems in the Netherlands (Frankena et al. 1991). The actual figures depend upon the method of assessment and most of these cases were not treated by veterinary surgeons but there is no doubt that lameness is often a severe welfare problem.

Clinical mastitis in mammals is a painful condition. The sensitivity to touch of the affected tissues (i.e. udder and teats) is clearly evident, particularly at milking time and there is obvious damaging of normal function. Mastitis incidence should have declined greatly with improved methods of prevention and treatment but it has not declined in the expected way, or has not declined at all (Barkema et al 1998, Schukken et al. 1998). In Denmark and in the Netherlands mastitis involving *Streptococcus uberis* or *Staphylococcus aureus* has not declined in incidence. Webster (1993) reports 40 cases of mastitis per 100 cows per year as an average for the UK

Other conditions of dairy cows which result in abnormalities of behaviour, emergency physiological responses, injury or impaired reproductive function also involve poor welfare. Reproductive problems in dairy cows have become very common in recent years with large numbers of cows being culled because of failure to get in calf (Esslemont and Kossaibati, 1997). Indeed culling policy has a significant effect on measurements of the prevalence or incidence of leg and foot problems, mastitis and reproductive disorders. Those farmers who cull at first signs of problems, or who cull at a fixed, early age will report fewer problems. The practice in the dairy industry is to cull at a considerably earlier age now than was the case 10

3.3 Milk yield and welfare in dairy cows

In 1999, the dairy cow may produce up to 18,000kg or more of milk per annum with a peak milk yield of 75kg per day and in several countries a mean of over 8000 kg per annum is obtained. This compares with UK figures of 6,000kg per annum and 30kg per day 10 years ago (Webster, 1993) and a beef cattle average of 1,000 — 2,000kg and 10kg per day. The dairy animal is producing considerably more than its ancestor would have. This raises questions concerning what is the maximum mean production level in a herd beyond which there will always be welfare problems.

The peak daily energy output of the dairy cow per unit body weight is not very high in comparison with some other species such as seals or dogs but the product of daily energy output and duration of lactation is very high. Hence long term problems are the most likely to occur (Nielsen, 1998). There are long term adverse consequences of high yield because, although some cows seem to be able to produce at high levels without welfare problems, the risk of poor welfare indicated by lameness, mastitis or fertility problems is greater as milk yield increases (Pryce et al. 1997,1998)

The steady increase in reproductive problems, some of which indicate poor welfare, as milk yields have increased is well known. As Studer (1998) states, "despite programmes developed by veterinarians to improve reproductive herd health, conception rates have in general declined from 55-66% 20 years ago to 45-50% recently (Spalding et al 1975, Foote 1978, Ferguson 1988, Butler and Smith 1989). During the same periods, milk production has greatly increased."

Studies showing that milk yield is positively correlated with the extent of fertility problems have come from a range of different countries (van Arendonk et al 1989, Oltenacu et al 1991, Nebel and McGilliard 1993, Hoekstra et al 1994, Pösö and Mäntysaari 1996, Pryce et al. 1997, Pryce et al 1998). Studer (1998) suggests that high producing cows which are thin, and whose body condition score declines by 0.5 to 1.0 during lactation, often experience anoestrus. A loss of condition score of about 1.0 during lactation was considered to be very frequent in the review presented by Broster and Broster (1998). Data on the relationships

between milk yield and reproduction measures from two large scale studies are presented in Table 1.

In some studies, effects of health problems on reproduction are evident, for example Peeler et al. (1994) showed how cows which were lame in the period before service were less likely to be observed as being in oestrus. Such lameness is more likely in high producing cows.

Direct links between level of milk production and extent of disease conditions are also evident from a range of studies, positive correlations being reported by Lyons et al (1991), Uribe et al (1995) and Pryce et al (1997, 1998 see Table 1). In addition to mastitis and leg and foot problems, which are often measured in such studies, the occurrence of other clinical conditions can also be affected by production level. Modern, high producing cows with good body condition have a high incidence of milk fever, retained placenta, abomasal displacement, metritis, fatty liver and ketosis (Studer 1998) and of digestive disorders (Seegers et al., 1997). The extended calving interval and the greater number of days to first service as milk production level increases (e.g. Table 1) could be related to a small extent to different management practices with higher producing cows but most of the effect is likely to be because there are more reproductive problems occurring in the higher producing animals and hence poorer welfare.

Table 1 :Relationship between milk production level and other variables in two studies. Correlation coefficients and standard errors
For all correlations p is less than 0.05 and for most it is very much less.

MEASURE	Pryce et al, 1997		Pryce et al, 1998	
Number of lactation records	33,732		10,569	
Calving interval	0.50	±0.06	0.28	±0.06
Days to first service	0.43	±0.08	0.41	±0.06
Mastitis	0.21	±0.06	0.29	±0.05
Foot problems	0.29	±0.11	0.13	±0.06
Milk fever	0.19	±0.06		
Somatic cell count			0.16	±0.04

Mastitis, foot disorders, reproductive disorders etc. occur more in higher yielding members of a herd irrespective of the mean yield of the group so it seems that the individuals which are working hardest metabolically in a group may be the most vulnerable.

The high yields of modern dairy cows are a consequence of genetic selection and feeding. Webster (1993) emphasised that ancestral cows were adapted to high fibre, low density diets. Despite changes resulting from breeding, most of the traits of the ancestor animals still remain. For example, cows do not adapt easily to high grain diets or to diets with high protein and low fibre (Webster 1993).

3.4 Conclusions

There is already evidence of welfare problems in dairy cows, for instance more than 50 cases of foot disorders and more than 40 cases of mastitis per 100 dairy cows can typically occur in Europe per year. Some of these animals and others in the herd may have reproductive disorders and other production related diseases.

There is clear evidence from several countries of significant positive associations between milk yield and mastitis, foot disorders, reproductive disorders and other production related diseases.

CHAPTER 4 HOW BST IS USED

4.1 The substance

Commercially produced BST is very similar to naturally occurring BST found in the bovine pituitary, with only a single amino acid difference or a few amino acid differences according to the manufacturers. It is produced by biotechnological methods involving the fermentation of *E. coli* strains containing the gene for the production of BST.

In the US it is estimated that 1.44 million cows were treated in the two year period from February 1994 to February 1996. Sales in the US are reported to have increased by 30% in 1997 over 1996. In 1998 over 100 million doses have been sold since it was commercialised almost 5 years ago. Thirty percent of the 9 million dairy cows in the U.S. are in herds supplemented with BST. A veterinary prescription is not required in the U.S.A. in order to obtain or administer BST.

4.2 The technique

Dairy cows are usually injected subcutaneously in the ischiorectal fossa (depression beside the tailhead) or behind the shoulder (post scapular). The volume of injectate of a commonly used formulation in the U.S.A. is 1.4ml.

The injection is typically repeated every 14 days.

4.3 Uses of BST

BST has been used for the following purposes;

- to increase milk production – in this case BST is given from the ninth or tenth week after calving until the end of lactation. In the US the generally claimed responses are from 2.25 l to 6.6 l of milk/cow/day.

- to extend the lactation of cows that would otherwise be culled because of inability to breed or other health reasons. BST can be used to keep a cow in production for 30 to 100 days extra.

These will permit a decrease in the number of cows necessary to produce the same quantity of milk.

The maximum increase in milk production occurs after three or four injections. The response to BST can vary from cow to cow. It is not possible to predict which cows will show large increases in milk yield in response to BST administration.

Manufacturers of BST list the conditions in which BST should and should not be used and the possible side effects of the treatment.

4.4 Conclusion

Commercially produced BST is very similar in structure to naturally occurring BST. It is recommended by a manufacturer that dairy cows should be given an injection of 500mg of BST once every 14 days.

CHAPTER 5 BIOLOGY OF BST ACTION IN DAIRY COWS

5.1 Introduction

Growth hormone (GH) is a component of a complex neuro-endocrine and metabolic system which maintains physiological homeostasis in the body. It is a protein composed of 191 or 190 amino acid residues and it is released from the anterior pituitary gland as four molecular variants: smaller fragments have also been reported. Pre-formed GH, stored in pituitary somatotroph cells, is released by exocytosis in response to several stimuli, including GH releasing factor (GRF) and somatostatin (SS) from the hypothalamus, blood concentrations of glucagon, insulin-like growth factors (IGFs) and oestrogen, and psychological stimuli, such as stress and sleep. Somewhat paradoxically, in view of the galactopoietic effects of increased blood concentrations of GH, low milk yields in underfed animals are associated with high concentrations of GH in blood (Bauman and Vernon, 1993).

Natural episodic release of GH from the anterior pituitary is chiefly controlled by the hypothalamic neuro-secretory peptides GRF (stimulatory) and SS (inhibitory), whose secretion into the hypothalamo-hypophyseal portal system is regulated by numerous neurotransmitters, including noradrenaline, dopamine and acetylcholine. Raised concentrations of GH in peripheral blood feed back onto the hypothalamus, inhibiting GRF and stimulating SS secretion, and these two peptides also exert acute negative feedback effects on the hypothalamus. In well-fed animals increased plasma concentrations of GH are associated with increased secretion from the liver of IGF1 and its binding proteins, and chronic inhibitory control of GH secretion is regulated by IGF1 feedback on central neural and hypothalamic systems (Prosser and Mepham, 1989; Burton et al, 1994; Etherton and Bauman, 1998).

Control of GH action on its target tissues is mediated by a wide range of factors, such as: concentrations in blood of hormones and metabolites; the type and level of blood plasma binding proteins; tissue distribution and concentration of GH receptors; and transmembrane signalling mechanisms. The major physiological actions of bovine GH (BGH) are to increase lipolysis, diabetogenesis, protein accretion, bone development, gluconeogenesis, mammogenesis and, in lactating animals, galactopoiesis.

5.2 Injection of exogenous GH (BST)

Based on the discovery in Russia in the 1930s that injection of extracts of anterior pituitary gland increased milk yield in cattle, the use of recombinantly derived BGH has now been established in several countries, most notably the USA. In Europe, it is more usually designated 'recombinant somatotrophin' - abbreviated to rBST or BST. Commercially produced rBST consists of a single molecular species which differs from pituitary (p) BGH by 0-9 amino acid residues (depending on the manufacturer). For example, the Monsanto product, Posilac, has double the potency of pBGH, from which it is immunologically distinct and exhibits several pharmacokinetic differences (Kronfeld, 1997).

Injected rBST also differs from endogenous pBGH in other significant ways, viz. i) blood concentrations are substantially higher than those achieved physiologically; ii) the pattern of release of slow-release preparations into the circulation differs markedly from the physiological pattern of episodic release; iii) feedback processes induce chronic inhibition of endogenous pBGH synthesis and secretion (Adriaens et al, 1995).

In principle, disruption of normal relationships between the elements of the neuroendocrine system described above by elevating supply of a *single* element of the complex might be expected to precipitate adverse effects, as for example in the human disease acromegaly, which is due to excessive secretion of GH from the pituitary. Despite this, some describe BST's galactopoietic action in cattle in ways which suggest the "orchestrated" enhancement of physiological control, e.g. " somatotropin is a homeorhetic controller that affects numerous target tissues in ways that are highly coordinated ....." (Bauman, 1992); (Etherton and Bauman, 1998). Strictly speaking, this is a misuse (or re-definition) of the term 'homeorhesis', which was introduced by Waddington in the 1950s to describe "an equilibrium (which) is not centred on a static state but rather on a pathway of (developmental) change" (Waddington, 1967).

5.3 Milk yield responses

Official estimates of the yield response to BST administration have varied from 10-25% (AHI, 1987) to 10-15% (CAST, 1993). However, responses can be variable and may depend on management factors to achieve a maximal response. Indeed, independent studies suggest that a third of treated herds will have less than a 10% increase (e.g. Chilliard, 1988), while there is at least one full report in which BST administration produced no significant yield increase (Kim et al, 1991).

In the USA, the Office of Technology Assessment (OTA) assumed a mean increase of 12% (approx. 5 kg/day), with variations being attributed to the quality of management (OTA, 1991). According to Etherton and Bauman (1998) "greater increases occur when the management and care of the animals are excellent". This claim might have some validity if it could be shown that high yielding cows prior to BST injection show consistently greater yield responses, but according to Kronfeld it is not sustained by examination of the literature (Kronfeld, 1994).

In low yielding cattle, dramatic effects on BST have been reported, e.g a 288% increase in yield in *Bos indicus* cows (Phipps et al, 1991) treated on days 75-95 of lactation, although between days 96 and 120 there was no significant effect on yield.

The production response increases with increasing dose of BST up to a maximum response at 30-40 mg/day (Bauman, 1992). The commercial preparation in use in the USA is a slow-release formulation in which 500 mg are administered every 2 weeks.

Although responses to BST are often described as 'smooth' (Bauman, 1992), periodic injections produce an unphysiological lactation curve. Thus, the results of Eppard et al (1991) show that the milk yield curve has a distinctly 'saw-tooth' appearance: during the 2 week period between injections the yield increased approximately 50% in the first 7 days, declining to baseline by day 14, before being sharply stimulated again by the next injection. In the case of 28 day injection cycles a lower than expected milk yield can be obtained in the fourth week (Vérité et al, 1989).

Claims for the increased efficiency of milk production when using BST, i.e. in terms of conversion of feed to milk, by means of lower maintenance costs per unit of milk produced. According to Kronfeld (1994), the claim may not apply for more than one lactation, particularly if a broader definition of efficiency, encompassing the lifetime performance of cows, is employed.

5.4 Milk composition

Significant effects on milk composition have been reported. For example, a decrease in casein concentration (mean 6.9%), which persisted over the 31 weeks of treatment, was reported by Kindstedt et al (1991). In this study of 26 Jersey cows receiving BST injections every two weeks throughout a complete lactation, casein expressed both as a percentage of total and true protein was significantly lower ($p<0.05$) than in the control group. According to the authors, at midlactation, concentrations of casein in the BST cows "decreased sharply and remained lower than the control group throughout the remainder of lactation". Following the same time course of change, nonprotein nitrogen expressed as a percentage of total nitrogen was significantly higher in the BST treated group ($p<0.05$).

Baer et al (1989) reported a sustained increase in long chain fatty acids (mean 11.5%) and a decrease in short chain fatty acids (mean 9.4%) over 28 weeks of BST treatment. Variations have also been described in response to a single injection of BST, e.g. milk fat increased by a maximum of 6% and milk protein decreased by the same amount (Chilliard et al, 1998) (See Figure 1). Somatic cell counts and IGF1 levels are also increased. Such changes appear to fall within the broad spectrum of concentrations which applies to milk of clinically normal cows as a whole (Kronfeld, 1994), although it is possible that BST could push concentrations of milk constituents beyond normal limits if they were already at those limits.

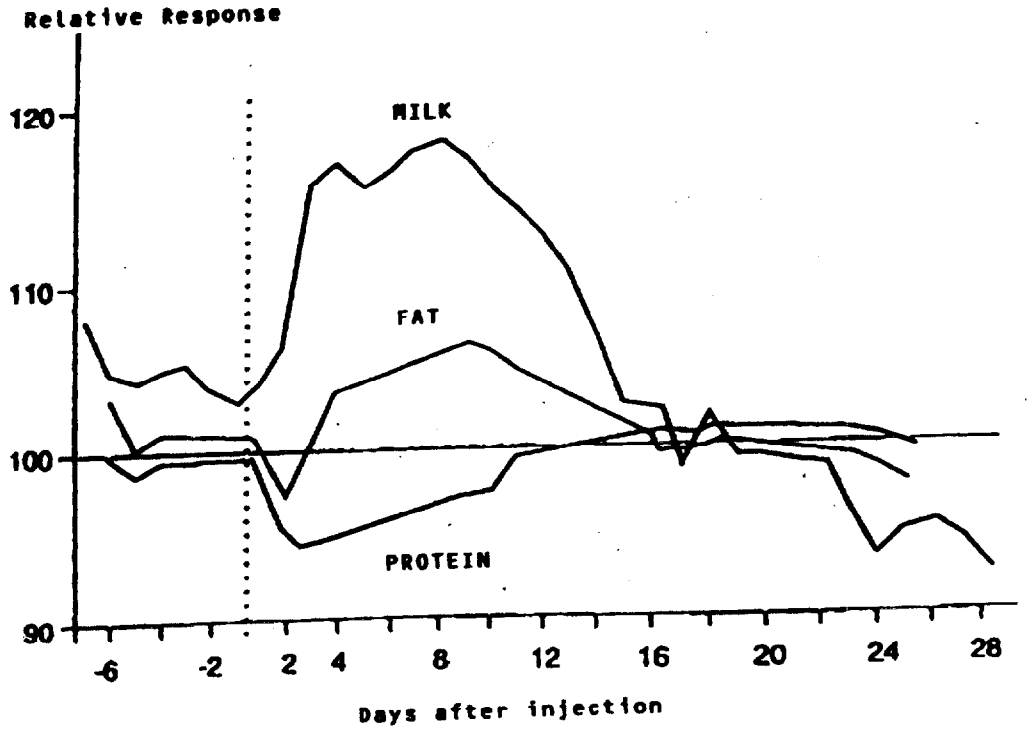

Figure 1:Changes in milk yield and composition following prolonged release somatotrophin injection (Verité et al. 1988)

Generally speaking, in the early phase of BST treatment, when the cow is in negative energy balance, milk fat concentrations increase and those of protein decrease, whereas these concentrations revert to normal as the cow attains positive energy balance (Bauman and Vernon, 1993).

5.5 Physiological actions of injected BST

Injection of exogenous BST is associated with marked elevations of circulating IGF1, small increases in thyroxine concentration and variable responses in circulating insulin, which may be related to blood sampling regimes or nutritional status (Prosser and Mepham, 1989).

As for other peptide hormones, the initial step of BST action involves binding with receptors on target tissues. GH receptors have been described on several cell types, e.g. hepatocytes,

adipocytes, lymphocytes, macrophages, fibroblasts, chondrocytes, □ islet cells and osteoblasts (Burton et al, 1994). There appear to be at least two classes of GH receptor in the bovine liver and hepatic production of IGF1 seems to be associated with the high-affinity receptor. There is much evidence that mammary tissue does not possess receptors for GH so that its galactopoietic effects are mediated largely by other factors (Etherton and Bauman, 1998).

Effects of BST can be considered under three headings: nutrient partitioning; cardiovascular effects; and alterations of mammary function.

5.5.1 Nutrient partitioning

When cows are treated with BST the increase in milk yield occurs very rapidly whereas the increase in voluntary feed intake is delayed until the 5-7th week of treatment. Thus, in the initial stages of treatment the requirement for extra nutrients to support lactation is met by mobilization of body stores or other tissues (Bauman and Vernon, 1993). Evidence that GH is instrumental in this process of nutrient partitioning is provided by studies which show that, in response to BST, mammary uptake of glucose and non esterified fatty acid (NEFA) is increased while that of muscle is reduced (Prosser and Mepham, 1989).

The lag in feed intake in the initial stages of treatment implies that the cows are in negative energy balance, and this is more marked when the yield response is greater. Eventually, as feed intake increases, the animal attains positive energy balance. Consequently, the adaptations in whole body metabolism which support the additional milk yield, and the factors which control these processes, must vary during prolonged treatment periods. This may account for the often conflicting reports of changes in circulating metabolite concentrations and in the concentrations of milk constituents.

Changes in fat content of milk are related to the potent effects of BST on adipose tissue. The response was formerly considered two-fold, i.e. decreased lipid synthesis (which thus 'spares' acetate and glucose) and increased lipolysis, releasing NEFAs (Bauman and Vernon, 1993) However, a more recent theory is that BST has no direct effects on lipogenesis or lipolysis but that it alters lipid metabolism on a chronic basis by reducing adipocyte sensitivity to insulin stimulation of lipid synthesis and increasing the responsiveness to catecholamine stimulation of

lipolysis (McGuire and Bauman, 1995). Recent data of Boisclair et al. (1997) has suggested that the elevated blood concentrations of NEFA observed in BST-treated heifers, and the marked elevations in NEFA in response to "intensive handling" of BST-treated steers, imply that BST sensitises adipose tissue to adrenergic stimulation. Whatever the ultimate explanation, the net result is that treated cows have reduced body fat and body condition. Generally, blood plasma NEFA concentrations are increased in cows in negative energy balance but do not change when they are in positive energy balance. When plasma NEFAs increase, milk fat concentration increases and the composition of the milk fat shifts to a greater content of long chain fatty acids, derived from the blood plasma.

Milk lactose concentration does not change appreciably in response to BST, due to the fact that, as the major osmole, it determines water flow into milk. The increased output of lactose is met by increased diversion of glucose to the mammary glands, and it has been suggested that this is effected by increased hepatic gluconeogenesis and decreased glucose oxidation in peripheral tissues (Prosser and Mepham, 1989).

5.5.2 Cardiovascular effects

There are several reports describing the increased rate of mammary blood flow in BST-treated ruminants, and the increased uptake from the blood of milk precursors appears to be partly accounted for by this increased flow (Fullerton et al. 1989). However, the precise role of the hyperaemic response remains uncertain. For example, it is unknown whether it is the cause or consequence of increased mammary activity.

Short term BST treatment has also been shown to increase cardiac output (Fleet et al. 1988); (Davis et al. 1988) and, in the few studies in which it has been recorded, heart rate is increased (Heap et al. 1989; Soderholm et al. 1988).

5.5.3 Alterations in mammary function

Evidence that BST affects mammary metabolism *per se*, albeit indirectly, is provided by studies of the mammary extraction of blood metabolites, i.e. by measurement of arterio-venous differences (□AV) across the mammary gland. For example, BST injections have been shown to increase mammary □AVs of glucose, acetate and triacylglycerols (Heap et al. 1989).

Strong evidence for BST-induced changes in mammary function is also provided by measurements of mammary blood flow. For example, in one study the pretreatment ratio of 'blood flow/milk yield' was about 700, whereas following BST treatment it decreased to 415 (Heap et al. 1989). This indicates that the extraction of substrates from blood perfusing the mammary gland increased substantially (although blood flow also increased).

Hence, effects on mammary tissue involve both increases in milk secretion rate per cell and increased maintenance of cell numbers (McGuire and Bauman, 1995).

However, by comparison with the large number of studies on BST aimed at assessing its galactopoietic effect there is a relative paucity of publications reporting the basis of its physiological action.

5.6 Mediation of effects by IGF1

The apparent absence of GH receptors in mammary tissue, and the lack of any galactopoietic effect when BST is infused directly into the mammary artery of lactating ruminants, suggests that alterations to mammary function are mediated by other factors. There is much evidence that in cows IGF1 performs this role (Prosser and Mepham, 1989, Burton et al, 1994).

Attempts to confirm this hypothesis by administration of IGF1 have been complicated by the fact that circulating IGF1 is largely (95%) bound to specific binding proteins (six in total), the major form of which has a molecular weight of 150 kDA. In treated cows, not only do blood concentrations of IGF1 increase but also that of IGFBP-3, while that of IGFBP-2 decreases. When animals are in negative nutritative balance, the effects of IGF1 are greatly reduced and

the galactopoietic effect impaired (McGuire and Bauman, 1995). GF1 may not act exclusively as an endocrine factor but also as an autocrine or paracrine factor (Prosser and Mepham, 1989), so that blood levels may reflect the cumulative production by different tissues. Nevertheless, the liver seems likely to be a major site of IGF1 production (Etherton and Bauman, 1998).

The galactopoietic effect of BST injections is accompanied by increased secretion of IGF1 in milk, which slightly precedes the increase in milk secretion rate (Prosser et al, 1991). Data on the magnitude of the increase in milk IGF1 concentration are sparse. The earliest report indicated a 3.7-fold increase as result of seven days of BST treatment (Prosser et al, 1989), while the Monsanto Company, in its submission to the European Community Committee on Veterinary Medicinal Products cited an "about five-fold increase" (CEC, 1993), but few reports have appeared in refereed publications and there have been questions about the accuracy of the IGF1 assays in some reports (Burton et al, 1994).

Direct evidence that IGF1 acts on mammary tissue is substantial. Thus: i) IGF receptors are present in mammary tissue and increase at lactogenesis (Burton et al. 1994); ii) IGF1 stimulates casein synthesis and glucose uptake in cultured mammary cells (Burton et al. 1994); iii) close unilateral intra-arterial mammary infusion of IGF1 in goats stimulated milk secretion to a significantly greater degree in the infused gland than in the non-infused gland (Prosser et al, 1990). IGF1 may also be responsible for the hyperaemic response to BST because the mammary blood flow of the infused gland was significantly increased by IGF1 infusion (Prosser and Davis, 1992).

According to Kronfeld (1994), many of the adverse health effects of BST are best viewed as a consequence of extending the phase of metabolic stress which normally accompanies the onset of lactation. Since the maximal response to BST is achieved within 2-5 days but the increase in feed intake takes 5-7 weeks to match the requirement for extra milk synthesis, the body goes into negative energy and protein balance, with associated changes in live weight, body composition and condition score. Consequently, BST administration extends the period of metabolic stress from 2-3 months to 4-6 months (see Figure 2).

As this situation differs from that in which milk yield has been increased by selective breeding

the often made comparison between yield increases due to genetic improvements and BST (Bauman, 1992) is of dubious validity. Thus, it has been claimed that pathological lesions evident in BST-treated cows are merely the result of increased yield. However, Kronfeld's analysis (Kronfeld, 1994) shows that, while milk yield increases with increasing BST dose up to twice the recommended commercial dose, there are continuing increases in the frequency of several lesions up to (at least) five times the commercial dose, viz. kidney cysts, lung-pleural adhesions, kidney fibrosis, muscle fibrosis and joint inflammation.

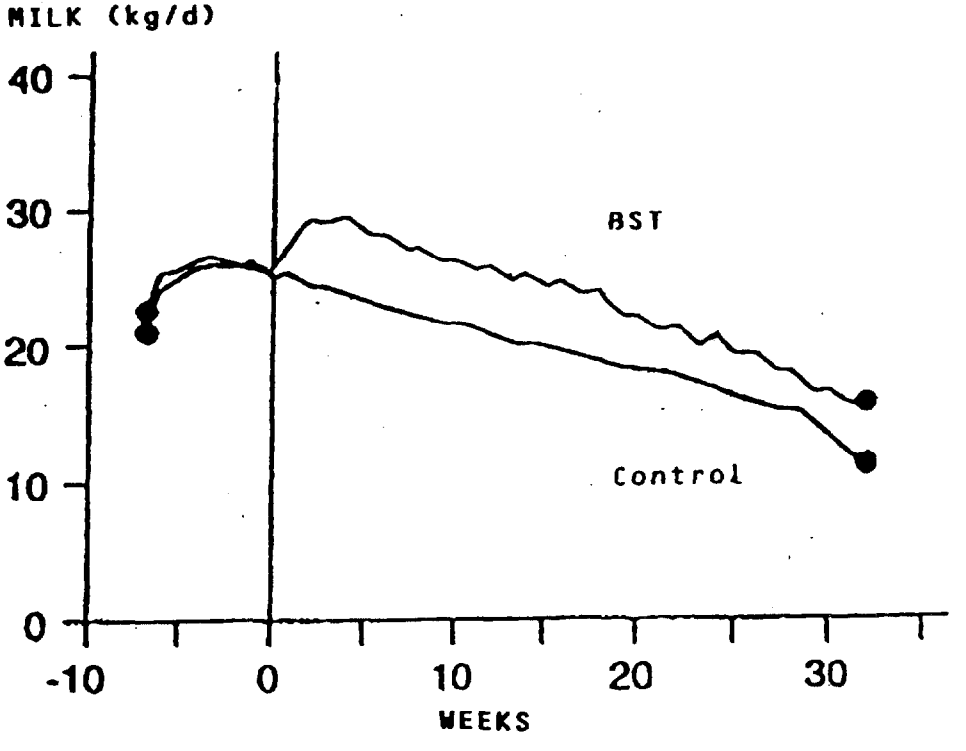

Figure 2: The lactation curve in cows receiving BST, first administered after the attainment of peak milk yield (Chilliard, Colleau et al, 1998)

5.6.1 Neurocrine and neuroendocrine actions of BST

The neural actions of GH were first documented in 1941, but these have been largely ignored until recently. Although pGH is synthesised principally in the pituitary gland, it is also now known to be produced at several ectopic sites, including the brain. GH receptors or binding

proteins also occur in the brain, where GH is involved in cell proliferation and maturation, neurotransmission and central behaviour. Consequently, as well as exerting endocrine effects "GH should also now be considered as a *bona fide* neuropeptide" (Harvey et al, 1993). Moreover, the occurrence of GH binding proteins throughout the pituitary gland and within pituitary cells implies that GH may have, previously unrecognised, endocrine, paracrine, autocrine and/or intracrine roles in hypophyseal regulation (Harvey et al, 1993.

In view of these neural actions of GH, the welfare implications of increasing blood concentrations of BST by injection would appear to require extensive investigation. Currently, there is a dearth of information on this aspect of BST's physiological effects.

5.6.2 Behavioural and other implications

There appears to be only a single refereed publication on the effects of BST on cow behaviour (Arave et al, 1994) - and that reports the frequency of various aversive behaviour patterns during implantation of a pelleted form of BST, rather than injection of the oil-based preparation which is used commercially. The 99 cows in the study were observed when they were implanted with 0, 120, 160, 240, 320 or 360 mg of BST. Flinching and lungeing were both observed in about 50% of cases and head-bobbing and a sagging of the back in 30-40% cases. Cows kicked at the handler or chute 11% of the time, and kneeling, indicating "extreme agitation", was observed in 5% of cases. Kicking, kneeling and ears back were significantly affected by BST dose. The extent of the swollen area around the implant was greater as implant dose increased. The implantation occurred in a handling chute and some behaviours decreased or disappeared with repeated implantations but others did not.

The fact that Boisclair et al (1997) reported that "BST caused a substantial rise in (blood) NEFA concentration ... when animals were subjected to intensive handling", suggests that, by sensitising adipose tissue to adrenergic stimulation, BST exacerbates the stress response. Whether this is merely a clinical response or has implications for animal welfare remains to be investigated.

Because of its anti-apoptotic effects, IGF1 could promote cell proliferation in cows to a stage of tumour neogenesis (see Report from the Scientific Committee on Veterinary measures in relation to Public Health). However, in general, cows on modern dairy farms do not live long enough for such effects to be of any significance.

There are other possible consequences of IGF1 which do not appear to have been investigated e.g. effects on calves in utero or feeding on milk containing high levels of IGF1.

5.7 Conclusions

The primary galactopoietic effect of BST in cows appears to be altered nutrient utilisation and mobilisation of non-mammary tissues, sparing nutrients for milk synthesis. This is achieved by effects on liver and adipose tissue but also by alterations in the responsiveness of other tissues to metabolic hormones.

BST increases cardiac output and heart rate and this is associated with an increase in the rate of mammary blood flow. Mammary metabolic activity is increased, involving greater substrate uptake and synthesis of milk-specific components. IGF1 seems to be largely responsible for such effects. In consequence, when BST is used, milk yields increase by about 10%, with compositional changes depending on the cow's energy status, e.g. IGF1 increases approximately five fold.

It appears that BST extends the period of metabolic stress which normally accompanies the onset of lactation. The cow remains in negative energy balance, utilising food reserves or other tissues, for some weeks after the commencement of BST usage.

The consequences of BST, acting as a neuropeptide, on the brain and on behaviour are not known.

Questions about the effects of elevated IGF1 levels in the cow on the welfare of the cow, or the welfare of the calf in utero, appear not to have been investigated. Neither have questions about the effects of elevated IGF1 levels in milk on the welfare of calves which drink the milk.

CHAPTER 6 BST AND MASTITIS

6.1 Introduction

The questions associated with the potentially increased incidence of clinical mastitis in BST treated cows and the resulting increased usage of antibiotics have been in the forefront of discussions for a long time. These issues have animal welfare aspects as well as public health aspects, and have been covered in previous reviews by various committees and organisations.

6.1.1 The European Union

In 1993 the CVMP (Committee for Veterinary Medicinal Products) as an advisory committee to the EU Commission issued final scientific reports on two applications for marketing authorisation of veterinary medicinal products containing bovine somatotrophin. In these reports the CVMP expressed the view relative to target animal safety of the products, that although the clinical trial data provided by the applicants shows an increased incidence of mastitis in treated animals as compared with the control animals this increase is an indirect effect resulting from the increased milk yield of the treated animals. It was furthermore recommended by the CVMP that, in order to take account of the prevailing practical animal husbandry conditions being less optimal than the conditions in the trial herds, the health and welfare of the target animals should be investigated in two-year post-marketing studies to include e.g. the incidence of mastitis.

At the end of 1994 the EU Council decided, however, to extend the moratorium on marketing and use of BST until the end of 1999. In 1998 a report by independent scientists should be prepared, "... in particular as regards the impact of the use of this product on the incidence of mastitis" (Council Decision 94/936/EC).

6.1.2. The situation in the USA

The mastitis issue has also been discussed relative to the US situation and by other international bodies. In 1993 the FDA decided to approve use of BST (POSILAC from Monsanto) on the US market effective February 1994. The documentation of the data behind the decision has been made publicly available through the Freedom of Information Summary (FOI) from 1993 (FDA 1993). Here data on mastitis is found in the section on Animal Safety (the data will be reviewed as part of the literature review) and in the conclusions on this topic the FDA sums up the facts as follows:

Use of BST increases:

- the risk of clinical and sub-clinical mastitis;
- the number of cases of clinical mastitis;
- milk somatic cell counts in some herds.

During the process the United States General Accounting Office (GAO) in 1992 called on the FDA to particularly study the potential risk to human food safety posed by a possible increase in drug residue in milk before approving the drug (GAO 1994). From the FDA FOI summary it appears that no animal welfare concerns were considered at all, and there was no mentioning of potential increase in antibiotic resistance caused by the increased use of antibiotics for mastitis treatment.

As a result of the FDA decision the label/package insert does contain a recommendation to precede the use of BST by the implementation of e.g. a comprehensive and ongoing mastitis control program, as well as a series of precautions and side effects including a section on mastitis, in which the FOI findings listed above are explained. However, animal welfare does not appear to have been an issue in the decision making process on BST in the U.S.A.

A post approval monitoring program (PAMP) was subsequently carried out by the company, to determine if mastitis incidence and antibiotic use was manageable under actual use conditions. The key components of the PAMP were the following three parts:

- A proactive system of collecting Adverse Drug Experience Reports
- A program of tracking milk residues by key dairy states before and after the approval
- A 28-herd study to evaluate the product under actual conditions of use.

A fourth part was designed to compare milk discarded from BST-using and non-using herds (Biotech Education 1998), but data from this part has never been reported, and the study was not mentioned in the final report.

The results of the PAMP (Monsanto 1996) will be reviewed in the literature review section.

6.1.3. The situation in Canada

Over the years there has been a great deal of debate over this item in Canada, including the mastitis issue. Recently, the Canadian authorities made a submission to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) meeting in 1998 which e.g. refers to the risk of antibiotic residues resulting from treatment of mastitis in BST cows and to the expression of the opinion that: " The greatest hazard is the emergence and spread of antibiotic resistant bacteria through the food chain, as an iatrogenic effect of treating mastitis in BST cows" (Canada, 1997).

In 1998 there was a report by scientists from Health Protection Branch, Health Canada which critically reviewed previous reports by Canadian authorities on the public health and human safety evaluations made. This included a conclusion that antibiotic resistance in farm-borne human pathogens associated with the increased risk of mastitis associated with the use of BST was not properly addressed so far, although it has obvious human health implications (Health Canada, 1998).

As recently as January 1999 the Canadian authorities finally decided, that BST should not be approved for use in Canada due to " a sufficient and unacceptable threat to the safety of dairy cows". This was substantiated by a scientific report from a committee of veterinary experts headed by an internationally recognised veterinary epidemiologist, in which increased risks of mastitis, infertility and lameness were found (Health Canad, 1999).

6.1.4. International organisations

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in their preliminary report on the 50th. meeting in February 1998 expresses the view that the risk of mastitis induced by BST is an issue of animal health that is not within the terms of reference of the Committee. However, the possible increased use of antibiotics was considered. This was done by strictly referring to the PAMP data from the US and the conclusions from this data, i.e. "that the use of BST will not result in higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for drug residues in milk could be managed by practices currently in use by the dairy industry and by following label directions for use" (JECFA 1998).

6.2. Mastitis in dairy cows

As already mentioned in Chapters 3, there are welfare aspects associated with high milk yield in dairy cows and the resulting higher risk of mastitis.

In this section some more details on the topic of mastitis in dairy cows will be presented as an aid in evaluating the importance of subsequently reviewed data on occurrence of mastitis in BST treated cows. Particular emphasis will be given to those items which seem important to the evaluation of animal welfare and public health aspects of mastitis in dairy cows.

6.2.1. General aspects

Mastitis is by far the most common disease of dairy cows. When veterinary surgeons describe the occurrence of clinical mastitis, they vary in the extent of clinical signs which must exist before they state that mastitis is present. In a precise study, the term clinical mastitis implies that there are signs of mastitis which can be detected by a veterinary surgeon conducting an examination of an animal. The prevalences of clinical mastitis reported in careful studies carried out in the EU have often been 40 or more cases per 100 cows per year, but with great variation between individual farms (Wilesmith et al. 1986, Plym Forshell et al. 1997, Schukken

et al. 1998, Seegers et al. 1998). Prevalence rates of sub-clinical mastitis vary even more between herds and also greatly depend on the methodology used in the diagnosis. Prevalence figures of around 50% of cows being clinically or subclinically infected are not uncommon in certain herds.

Mastitis is also the most costly disease to dairy farmers, and the number one cause of antibiotic use in dairy cows in spite of the fact that current treatment protocols are not necessarily clinically or cost effective (Radostits et al. 1994, Sandholm et al. 1995, Leslie and Keefe 1998).

Mastitis is an inflammation of the mammary gland, characterised by increased somatic cell counts (SCC) in the milk and by pathological change in the mammary tissue. The disease is usually caused by pathogenic micro-organisms entering the gland through the teat duct. Many different bacteria cause mastitis, some being considered as specific udder pathogens, others being merely opportunistic organisms that cause disease when there is a increased susceptibility of the udder for some reason. Among the common bacteria causing clinical mastitis are *Staphylococcus aureus*, *Streptococcus* spp., *E. coli*, as well as other minor and major pathogens (Bramley 1992, Wilesmith et al. 1986).

Mastitis has been described as being of different types, although the nomenclature is neither exclusive nor necessarily standardised. Furthermore, individual cases tend to quickly develop and thereby change between the categories, which is also why classification of cases and statistical data may be difficult to compare across different studies, unless a common protocol has been used.

The following general classification system exists for different types of mastitis:

- ***sub-clinical***
- ***clinical***
    - ***chronic***
    - ***acute /hyper-acute***
        - ***mild or severe***

Sub-clinical mastitis can only be detected by the application of some sort of diagnostic test to a milk sample. The tests used are either tests aimed at directly revealing the micro-organisms involved or indirect tests to present evidence of inflammatory reactions in the udder tissue and/or milk. Somatic cell counts (SCC) may be considered among the latter, although increased cell counts may be caused by physiological processes, which are not inflammation due to infection by micro-organisms (Coulon et al., 1998). In any case increased SCC becomes a quality issue, since SCC standard values are used in quality and price evaluation of milk delivered for consumption. .

Clinical mastitis exists when a cow shows clinical signs of udder infection in one or more of the quarters. The different types of clinical mastitis mentioned in the following may sometimes be seen as different phases which occur when the characteristics of a case change over time.

Chronic mastitis often involves an insidious appearance of long duration, which gradually leads to morphological changes in the udder (fibrosis, change in size or shape). ***Acute /hyper-acute*** generally refers to a sudden onset of signs.

Mild cases merely show changes in the milk (flakes, clots, watery appearance) (Grade 1 cases). ***Severe*** cases show clinical signs of inflammation in the udder (heat, swelling, pain, etc) (also called Grade 2 cases) and sometimes even fever and depression in the cow (Grade 3 cases).

6.2.2. Animal welfare aspects

As mentioned in Chapter 2, clinical mastitis is a painful condition, at least in the proportion of cases which has been referred to in 6.2.1. as *severe acute clinical mastitis*. This category is defined by the local reaction in the udder including pain, and in some of these cases, fever and depression would add to the distress of the affected animal.

Unfortunately, very few reports are available on the distribution of acute clinical mastitis cases between the *severe* and the *mild* categories. Wilesmith et al. (1986), defined *mild cases* as those involving milk or quarter, while *severe* and *very severe* were used for defined degrees of systemic disturbance. They reported that 58 - 62% of the clinical cases were *mild* over a three year period. It should be noted, that according to these definitions, an unknown proportion of the classified mild cases could have had some pain and discomfort due to local reactions in the affected quarter, while probably the large majority of the classified severe cases had experienced such or more likely more pronounced pain and discomfort. There was a fatality rate of 0.3 - 0.6% among the cases of clinical mastitis. The annual incidence rates were 25 - 31% of cows affected, but with 1.5 - 1.6 cases per cow per year for a total of 41 - 55 cases per 100 cows per year. They comment that their results suggest that severe cases have been more common in recent years, possibly due to an increase in the proportion of clinical cases due to *E. coli*. Further work in the UK (Blowey and Edmondson 1995) on the economics of mastitis assumes a proportion of mild cases to be 70% with reference to previous UK studies (refs. to be given later).

Qualitative information on pain and discomfort associated with clinical mastitis is very scarce. Alban (1995) in a subjective ranking of cattle diseases according to their presumed welfare consequences scores clinical mastitis as having on average a moderately painful character. In a subsequent paper by Alban and Agger (1997) discrimination between the various types of mastitis gives different scores for pain, ranging from "very painful" in necrotising mastitis to "minor pain" in mild mastitis.

Hillerton (1998), in promoting the needs to treat clinical cases with antibiotics in spite of current efforts to reduce the amount of antibiotics used in animal production, states that: "Mastitis is a painful condition causing moderate to severe distress" and "Primary consideration is that all animals with clinical mastitis are suffering".

The classification of pain associated with clinical mastitis is being applied by Alban (1995) and Alban and Agger (1997) in the further characterisation of welfare associated with disease according to the notion that also the duration of the disease episode is important. This model refers to earlier work by Morton and Griffith (1985) and by Willeberg (1991).

The duration of cases of clinical mastitis obviously varies, but the acute episodes which are most relevant to welfare considerations are on average measured in days (Alban 1995). In many of the cases there will be a gradual recovery during the course of the episode, so that the pain and discomfort will decrease throughout the duration of the episode. On the other hand, fatal cases will deteriorate with progressively poorer welfare throughout the course of the episode.

Statistical data on the duration of cases of clinical mastitis in dairy cows are not readily available outside of controlled studies such as those later reviewed on the use of BST. Such data will therefore appear as results for the untreated control groups from those studies that reported such results (see Section 6.3).

The importance of the incidence of clinical mastitis in dairy cows to the assessment of the welfare consequences of this condition has already been highlighted in the previous sections of this chapter, as well as in Chapters 2 and 5. The more formal presentation of the arguments for this importance can be found in Willeberg (1991), who expressed the welfare importance of disease as a function of its incidence, duration and the intensity of pain or discomfort. The incidence of the disease in a population of animals must also be taken into consideration. Clearly, the more frequently a disease condition occurs in a population, the more important is this condition to the overall welfare of animals in this population.

Although sub-clinical mastitis does not *per se* cause pain or discomfort for the cow and therefore has no direct welfare consequences, it is generally thought that cows with sub-clinical mastitis are at higher risk of getting subsequent episodes of clinical mastitis.

6.2.3 Treatment and prevention of mastitis in dairy cows

Treatment of clinical mastitis cases with antibiotics is not limited to those cases which according to the previous classification may be classified as *severe*, although such cases are probably more likely to receive systemic treatment. Also *mild* clinical cases are often treated with local application of antibiotics, such as intra-mammary tubes. Even cases of *subclinical mastitis* are sometimes treated with antibiotics, depending on other factors in the herd. Cows

are often treated on being dried off before calving (Radostits et al. 1994). The result is that mastitis in dairy cows is associated with a very large usage of antibiotics.

6.2.3.1. Antibiotic resistance

The possible effects of residues in milk on human health are discussed in the report of the Scientific Committee on Veterinary Public Health. Antibiotic resistance may have important consequences for farm animals. Microbial resistance to antibiotics could result in less effective control of disease in cattle and other species and hence lead to poor welfare and increased costs for farmers.

6.3 Comparative studies on mastitis in BST treated and non-treated cows

This section will describe the results of studies aimed at documenting if and how mastitis aspects may differ between BST treated and non-treated cows. A large number of studies have been carried out, which among their primary or secondary aims have had such aspects, being either qualitative or quantitative or both.

It should be noted here, that due to missing detailed identification of individual studies and to the nature of some of the reports being reviewed in the following sections, it is not possible to ensure that data from a study do not appear again as data in other reports, especially when it comes to the meta-analyses. This will unintentionally cause some non-independence among results presented in different reports including this report.

6.3.1. Qualitative aspects

6.3.1.1. Types of clinical mastitis

There is no information from the available comparative studies to describe changes following BST treatment in the proportional composition of clinical mastitis cases with respect to type, i.e. acute *versus* chronic and mild *versus* severe based on the clinical signs.

6.3.1.2. Microbiology

6.3.1.2.1. Clinical mastitis

In the Technical Manual on Posilac (Monsanto 1993) a summary table of microbiological findings from 10 comparative studies is presented. From this table it appears that *Staphylococcus aureus* and coliforms are relatively more frequently isolated from clinical mastitis cases among the BST treated cows than among the control cows (18% *versus* 11% and 26% *versus* 19%, respectively). The Manual concludes that the relative distribution is not affected by the treatment but no statistical data were presented. Cole et al. (1992) also found these two groups of pathogens to account for the majority of cases.

Pell et al. (1992) described a herd of Jersey cows in which chronic cases of clinical mastitis caused by *Staphylococcus aureus* occurred among the BST cows but not among the control cows. In the study by Weller et al. (1990) *Streptococcus uberis* was the most common bacterium isolated. The paper by White et al. (1994) mentions that microbiological identification was not uniformly determined at all trial locations, and data were not summarised. In Judge et al. (1997) fewer isolates of *Staphylococcus aureus* and coliforms were found among clinical mastitis cases in treated than in controls, while *Streptococcus* spp. were more frequent in the former than in the latter group.

6.3.1.2.2. Prevalence of sub-clinical infections

McBride et al. (1988) showed results indicating that the prevalence of infected cows was significantly greater in mid-lactation in BST-treated compared to control cows. Lissemore at al. (1991) observed a higher prevalence of infected cows and quarters in mid-lactation in BST-treated compared with control cows. Both these studies used different dosages of BST and the differences were most apparent for the high dosages.

McClary et al. (1994) found only few differences among the bacteria isolated from sub-clinical mastitis cases (so-called IMI: Intra Mammary Infections) between treated and non-treated cows. Only for *Staph.* spp. were there more cases in treated cows than in controls.

The FOI-summary indicates that sub-clinical mastitis identified by growth of bacteria from milk samples showed at least 50% excess risk in BST-treated cows. These differences were statistically significant. The difference appeared to be caused by differences originating in the bacteriological sub-groups of "pathogen" and "coagulase negative *Staphylococcus*".

6.3.2. Quantitative aspects

In this section duration and incidence of mastitis from comparative studies will be reviewed. It is important to note, that since BST is most often administered only in part of the lactation period (i.e. from approximately 60 days after calving to dry-off), incidence figures will implicitly refer to this period of risk. If such a figure is compared with an incidence based on the entire lactation period, the former incidence will of course tend to be lower than the latter, if such were available. For the same reason, control cows from BST studies will show an incidence of mastitis which is lower than that of a "normal" non-treated cow for an entire lactation period. Due to the higher risk of mastitis in the first 60 days of lactation, the risk for the remaining part of the lactation is probably only about half of the total lactation incidence.

6.3.2.1. Duration of clinical mastitis

The duration of episodes of clinical mastitis is important for at least two reasons, for the impact on welfare of the cows and for the total use of antibiotics in the treatment of cases.

McClary et al. (1994) found no difference in duration between treatment groups, and Judge et al. (1997) found no difference between treated and non-treated cows in the average number of days for which milk was discarded when antibiotics were used (10.0 and 11.5 days, respectively).

In his review of the literature, Kronfeld (1994) found three studies with strong evidence for a prolonged duration of clinical episodes in treated over non-treated cows. One of these reports (Thomas et al. 1991) was based on 871 cows from 15 herds and this report shows that the proportion of cow days with antibiotic treatment for clinical mastitis in BST treated cows were more than twice that in non-treated cows (0.36 % versus 0.16%). Average case length also varied considerably in the study of Cole et al. (1992), but no consistent pattern was apparent. The third study reviewed by Kronfeld (1994) is that of Pell et al. (1992) in which, on average, control cows were treated for clinical mastitis for 1.5 days, while BST cows were treated for 8.9 days. This was probably confounded by the problem of chronic infections by *Staph. aureus* mentioned above. Burton et al. (1994) reported that the total number of treatment days for mastitis were close to three times higher in BST treated than in control cows. In the FOI summary there was no difference in the average number of days affected between BST and control cows with clinical mastitis, but there was a significant difference in number of days affected per 252-days lactation periods between treated and control cows due to the increased risk of clinical mastitis in BST treated cows. In the PAMP study no difference was found. In general, the most substantial studies on the duration of treatment for mastitis indicate that this was greater after BST usage but not every study showed this effect.

In experimental studies by Vandeputte-Van Messom and Burvenich (1993) BST was shown to influence the recovery after experimental coli-mastitis. Recovery was measured mainly in terms of return to milk production. There was better recovery in some BST treated cows, but not in others. The effect was found both when BST was given before and after the onset of infection.

6.3.2.2. Incidence of clinical mastitis

Reports published since the 1980's on the efficacy of BST in increasing milk yield have often had as a minor secondary aim to evaluate any adverse health effects of the treatment. Only a limited number of these reports, however, have documented their findings with actual numerical information, while most have merely commented, that no obvious health problems were observed. Given the often small number of cows in these studies, such undocumented statements are of little value.

Published reports up through 1991-92 containing actual data on cases of clinical mastitis have been reviewed by Willeberg (1993). In the review data from 11 individual studies and from 6 meta-analyses of series of studies were analysed. The data from individual studies illustrate the wide variability in the ratio of the risk of clinical mastitis in treated and non-treated cows from individual herds in which BST was used , ranging from 0.36 to 1.8. In meta-analyses the ratio varies between 1.17 and 1.47. The difference between the two series of estimates are due to the large sampling variability in the studies based on small numbers of cows as well as the variability in risk between individual herds, which is averaged in the meta-analyses. It was concluded, that the more reliable estimates from the meta-analyses indicate that BST treatment results in an excess risk of clinical mastitis of 15-45 % over that in non-treated cows, that this effect may be partly due to an indirect effect through increased milk yield, and that this increase is of concern regarding the welfare of future populations of dairy cows.

Since this review a number of relevant publications have become available. In a general review, Bauman (1992) supports the observation that data from many cows are needed to substantiate what he calls a "subtle health effect", and he cites only the study by Phipps (1989), which claims no observed effect in a summary of data from 1300 cows. However, these data were re-analysed in two of the meta-analyses in Willeberg (1993) with resulting estimates of excess risk of 27 and 47%, respectively.

Pell et al. (1992) observed an increased number of cases of clinical mastitis in BST treated cows in a study of only 46 cows, but came to no conclusions due to the small number. Data from more than 600 cows (FDA's POSILAC Freedom of Information Summary –FOI-, 1993) enabled the estimation of a statistically significant 79% excess risk of clinical mastitis in BST treated cows compared with non-treated cows, when analysed by the same meta-analysis technique as used by Willeberg (1993). Also sub-clinical mastitis was shown to be significantly more common in treated cows than in controls, as well as treated cows having an increase in the number of somatic cells in the milk (SSC). No mention was made of any animal welfare concerns. Hansen & Otterby (1993) in a short review indicate that the risk of clinical mastitis may be increased in BST-treated cows.

White et al. (1994) made meta-analyses of data from a number of individual studies, and estimated the excess risk of clinical mastitis in treated over non-treated cows to be 42%. In logistic regression analysis, however, the introduction of milk yield as an explanatory variable caused the association between BST and clinical mastitis to become non-significant. Based on these data the authors conclude that the excess clinical mastitis is an indirect effect of BST mediated by the increased milk yield, which is regarded as a direct causal factor. McClary et al. (1994) found no effect of BST on clinical and sub-clinical mastitis in a study of 352 cows during one lactation, while there was an increase in the SCC. Neither could Hansen et al. (1994) demonstrate any increased risk of clinical mastitis in another study on 352 cows over two lactations. Burton et al. (1994) in a review concluded that there may be an apparent adverse health effect of BST treatment in the case of clinical mastitis, since some studies have found an increase in cows treated with higher doses or over multiple lactations.

In the post-approval monitoring program (PAMP) of POSILAC an evaluation of clinical mastitis in 28 herds was performed (Collier 1996). The study confirmed the occurrence of a statistically significant increase in clinical mastitis in BST treated cows, although at a lower level (23%) than at the FOI estimate described above (79%). Judge et al. (1997) reported a 22% non-significant overall increase in risk of clinical mastitis in a study involving 555 cows from 4 herds. However, very marked herd differences were apparent, so that in one herd there was a statistically significant increased risk of 330 % in BST-treated cows. However, the mastitis incidence in control cows from these herds was low compared to reported average values, which could make the results less representative for herds of average background risk of mastitis. Fontes et al. (1997) reported on 58 Brazilian cross bred cows and found a tendency to more mastitis in BST-treated cows.

Kronfeld (1997), in a review of some of the published studies as well as the FOI and PAMP reports, criticises the apparent inconsistencies and weaknesses in the reports on clinical mastitis, and he also points to the animal welfare aspects of the continued use of BST in spite of the controversy over interpretation of the published data. Ruegg et al. (1998) reported on culling rates in 19 herds using BST and they found no statistically significant increase in overall culling over that in 13 non-BST herds. However, they do report higher proportion of culling due to mastitis in BST herds compared to controls, but this was not significant due to [illegible] of the statistical testing of the small number of herds. In a recent Canadian review

(Health Canada 1999) the conclusion of several meta-analyses was that there was an increased risk of clinical mastitis by about 25%.

6.3.2.3. Sub-clinical mastitis

Since sub-clinical mastitis can be diagnosed only by testing of milk samples the measures of the frequency of sub-clinical mastitis are technically speaking prevalence figures. When SCC is used to indicate sub-clinical mastitis the results may be presented either as prevalence of high somatic cell counts or as average SCC for the cows in the group.

6.3.2.3.1. Somatic cell counts (SCC)

McBride et al. (1988) showed that the mean SCC was significantly greater throughout the treatment period in high-dose-BST-treated compared with control cows. Peel et al. (1988) found that the SCC was significantly increased in BST treated cows in two out of eight studies reviewed; in five others the SCC were non significantly elevated and in one it was non significantly lowered.. Craven (1990) observed a statistically significant increase in SCC towards the end of the lactation period in some locations. Lissemore at al. (1991) observed a higher SCC for some months in BST-treated compared to control cows. Thomas et al. (1991) found no differences in SCC during treatment. In the study by Cole et al. (1992) the levels of SCC generally reflected the level of clinical mastitis, which increased with increasing dosage of BST. Some of these studies used different dosages of BST and differences were most apparent for the high dosages.

McClary et al. (1994) found increased SCC in BST-treated cows with a significant dose-response trends for both primiparous and multiparous cows. White et al. (1994) found only slight associations between treatment and SCC. Masoero et al. (1998) found no effect of BST on SCC in BST-treated compared to control cows. Similar conclusions were obtained by Monsallier (1991).

Millstone et al. (1994) published results from meta-analysis of data from 8 studies, and the results indicated a statistically significant increase of 19% in mean SCC in BST-treated over

control cows across the 8 studies. In 3 individual studies there was a significant difference, while 5 studies showed insignificant differences.

The FOI (1996) found that SCC were elevated in some herds when BST was used. It is possible that this was due to higher sub-clinical infection rate in these study locations. In the PAMP study there were no significant differences in SCC. A similar conclusion was reached in the Canadian review (Health Canada 1999), although tendencies were found in some instances.

In general it appears that cell count data does not give reliable information about BST effects, but where there are differences, the SSC was found to be higher in BST treated animals.

6.4. Discussion of epidemiological issues in the studies reviewed

A number of epidemiological issues can be raised relative to the field studies of BST which form the basis for the animal safety evaluations by the various agencies involved in the scrutiny of the product as part of the authorisation for marketing (Willeberg 1993, 1994 and 1997). The following epidemiological points will be considered relative to the incidence of clinical mastitis. However, the general principles here are also relevant to studies on lameness and fertility problems.

- sample size and resulting power of the individual study to identify excess clinical mastitis due to BST treatment;
- importance of different mastitis rates during the pre-treatment period between cows belonging to the BST group and to the non-treated group;
- herd effects and representativeness of experimental herds;
- relevance and correctness of the "indirect effect through milk yield" explanation.

6.4.1 Sample size and resulting power to identify treatment effects

Willeberg (1993) has dealt with this issue extensively. The point to be made is that the many published papers on individual BST studies, which typically include 40 - 60 cows in each of the treatment and non-treatment groups, have far from sufficient statistical power to detect a realistic difference in the risk of clinical mastitis between the two groups. Assuming a base-line risk of 20 cases per 100 non-treated cows for the relevant part of the lactation period and hypothesising an increase by 35% in this risk from BST, it would require approximately 600 cows in each group for this difference to become statistically significant (95% confidence level and 80% power). Consequently, the great majority of single study reports conclude, that there is no significant increase in clinical mastitis due to BST. However, the absence of significance is often the result of a low sample size. Subsequent meta-analyses have corrected for this problem of low power, and consequently estimates of increase in risk ranging from 17% to 47% due to BST treatment were obtained (Willeberg 1993).

In a previous paper the issue was dealt with indirectly by pointing out, that examination of "subtle health effects such as mastitis incidence" will require large number of animals treated for several lactations under a range of environmental and management conditions (Eppard et al. 1987). In the paper by Millstone et al. (1994) a similar discussion has been presented with respect to mean SCC figures from individual BST studies.

The point made above concerning studies of the effects of BST treatment on the incidence of mastitis is also relevant to other causes of poor welfare such as foot disorders (Chapter 7), reproductive disorders (Chapter 9) and to health in general and welfare in general. It is not possible to conclude whether or not BST treatment affects the incidence of problems unless a sufficient sample size is used. Some published studies and other reports have concluded that BST had no effect on disease incidence or other indicators of welfare when the data sample was insufficient to allow such a conclusion.

6.4.2 Different pre-treatment mastitis rates in the BST group and the non-treated group

Phipps (1989) comments on the fact that: "in certain circumstances there appears to be an increased incidence of clinical mastitis in treated cows yet in other cases there is no indication of increased clinical mastitis as a result of BST treatment". He goes on: "However, the overall incidence of clinical mastitis was notably also higher before BST treatment commenced in cows already allocated to the treatment group and thus the relative incidence of mastitis was not affected by BST treatment".

Also White et al. (1994) found that the mastitis incidence during the pre-treatment period was significantly higher in the to-be-treated group than in the to-be-non-treated group. This paper suggests that this may be due to a greater predisposition to mastitis in the treatment group than in the non-treatment group, i.e. the randomisation procedure used in allocating the cows to either group had not been successful on this point. Nevertheless, in White et al. (1994) the statistical analyses of the treatment effects were carried through ignoring the potential bias introduced by this unfortunate event. This appears to be a case of "randomise and close-your-eyes", i.e. to rely on the supposedly beneficial effect of randomisation even though the data itself shows that the randomisation procedure had failed on a critical point.

In important studies such as these for resolving the controversy over the BST-mastitis issue the scientifically most sound solution might have been to analyse separately the information from those herds or individual studies with no differences in pre-treatment mastitis rates. This, however, was not attempted.

6.4.3. Herd effects and representativeness of experimental herds

The incidence of clinical mastitis varies greatly among dairy herds, and consequently published meta-analyses (Phipps 1989, Craven 1991, Thomas et al. 1991, White et al. 1994) contain evidence of a considerable herd effect in terms of differences among herds in the risk of clinical mastitis in non-treated cows, and in differences in the risk ratios between treated and non-treated cows. Adjustment for these herd effects, however, was not always made in the

published analyses and no information was given on how representative the selected herds were for the population of potentially BST-using herds. Neither has any formal study been made to identify factors which may be responsible for these differences in the effect of BST among herds.

Furthermore, some reports mention the need for larger field studies to be carried out under a range of environmental and management conditions in order to detect "any subtle health effects" (Eppard et al. 1987, Bauman 1992). The Committee on Veterinary Medicinal Products (CVMP) advising the European Commission said in its final report on two BST applications that it is important to verify that the overall level of risk to the health and welfare of the target animal is not increased when the product is used under practical farming conditions where standards of animal husbandry may not be as high as those in the experimental herds. The CVMP recommendation is therefore that, if BST should be allowed in the EU, then a wide-ranging study of at least two years duration should be undertaken to determine the effects of BST on the incidence of mastitis and associated metabolic disorders under practical conditions of use (European Commission 1993).

The argument that the excess risk associated with BST is of no public health concern because it is smaller than the variation caused by herd effects and other factors such as season (FDA 1993) does not hold for animal welfare concerns. Antibiotic residue avoidance programs were stated to be adequate to detect and prevent the potential public health effects of treatment, but no additional safeguard exists to prevent animal suffering in clinical cases of mastitis. Therefore, all factors which decrease the risk of clinical mastitis are relevant as potential preventive measures to improve animal welfare. The main issue in choosing among them is the possibility of managing the exposure to the respective factors. While one has full control over whether or not to use BST, in practice very little control can be exerted over seasonal and herd factors, as long as the causal factors behind their effects have not been identified in more detail.

6.5. Conclusions

Clinical mastitis is often a painful disease. The welfare of most cows with mastitis is poor, the extent of poor welfare being dependent on the severity of the condition.

It has been stated in certain published papers and reports that BST has no effects on some welfare measures e.g. mastitis, foot disorders, health in general, or welfare in general. However, in many cows the sample sizes used were too small to justify such conclusions and meta-analyses have revealed that there are effects.

The duration of episodes of treatment for clinical mastitis were longer in BST-treated than in non-treated cows.

BST usage increases the risk of clinical mastitis above the risk in non-treated cows. The magnitude of this increase has been variously estimated by meta-analyses or large scale studies at 15- 45%, 23%, 25 %, 42% and 79%.

These estimates describe an increase due to BST which is not only statistically significant but also biologically relevant and of considerable welfare concern. Whether this effect is direct or indirect does not alter the welfare concerns.

CHAPTER 7 EFFECTS OF BST ON LEG AND FOOT DISORDERS (LAMENESS)

7.1 Introduction

Lameness in dairy cattle has been considered as one of the major causes of poor welfare and economic losses in dairy farming. As explained in section 2.2, assessment of leg and foot problems is not always straightforward. One of the major problems is that in order to get a proper insight into the prevalence and incidence of claw disorders in particular, one has to lift the feet and examine them thoroughly.

The effect of BST on health of dairy cattle has been scrutinised for years now. No direct acute toxic effects of BST on the claws or legs of dairy cattle have been described. There are few planned studies on the effects of long term administration of BST on the incidence or prevalence of foot or leg disorders.

7.2 Foot and leg disorders

A possible association between BST treatment and an increased incidence of lameness has been reported by several authors (Zhao et al.,1992; Cole et al., 1992; Kronfeld, 1997; PAMP, 1996). Cole et al. (1992) described a higher incidence of clinical lameness in the BST treated animals in the first and second year of the BST administration. In the high treatment group (3.0g/14 d) lameness had a more chronic character. Lameness was diagnosed by clinical daily health observations. Clinical lameness diagnoses included foot rot, hock problems, sole abscesses, lameness due to injuries, lameness due to limb and joint problems such as swelling of the foot, hock, knee or leg, and "undiagnosed". The results did not give an explanation of the different lameness diagnoses. During the study, animals were kept in tie stall confinement housing. The housing system might explain in part the overall low incidence of claw disorders compared with studies where dairy cattle are housed in a loose housing system. Wells et al. (1995) described the long term effect of the administration of BST in 94 pairs of high producing cows The prevalence of gait abnormalities and visual evaluation of the limb was estimated at a single farm visit, but the feet were not lifted. A high prevalence of lameness was recorded, 39.4% of untreated animals and 46.9% of BST treated animals ($p > 0.05$). Limb lesions significantly associated with long term BST treatment were superficial laceration of the tarsus, superficial swelling of the metatarsophalangeal joint. In

this study treated animals had a lower risk for femoral lesions and superficial lacerations. Kronfeld (1997) described the results of a FDA-PAMP study. Kronfeld emphasised in particular the high incidence of laminitis in treated cows. This high incidence of laminitis has been attributed to diet i.e. the use of more grain to increase energy density aid minimise loss of body condition. PAMP data (Monsanto, 1996) indicated that cows injected with BST had approximately 50% more days observed of foot and leg disorders. There was an association between the use of BST and incidence and duration of hock disorders, knee calluses and lesions of the foot. These were primarily associated with lacerations and bruises associated with infections. These observations were also associated with altered gait. Sample size, definition of diagnoses and pre-treatment incidence rates of several foot and leg disorders might have influenced the outcome of this study. As a consequence some possible associations between the increased incidence of, for example, foot rot and laminitis and the use of BST were not significant. The PAMP data are summarised in Table 2 below. The FOI summary for BST showed the same association between the use of BST and an increased incidence and duration of knee calluses, hock disorders and foot disorders. More multiparous treated cows were lame and suffered over a longer period of time. Pell (1992) and Oldenbroek (1990) could not find an increased incidence of lameness associated with the use of BST.

7.3 Skeletal and joint problems

Cole (1992) described a slight increase of femur length associated with BST treatment in primiparous cows. Several reports of the same or similar study have been presented. Cole (1992) described that the incidence of macroscopic and microscopic lesions of bone and cartilage was unaffected by BST treatment. However no data were presented. The FOI summary part 3 indicated that post-mortems of five cows, that were chronically treated with BST 500 mg/14 days, revealed that in all animals multiple articular (subchondral) erosions and other joint pathologies were observed in multiple joints. However the authors concluded that environmental factors might be responsible for the articular lesions rather than any direct effect of BST.

The PAMP study carried out in the USA has provided clear evidence for the effects of BST treatment on foot disorders and other musculo-skeletal problems. Data were collected on a daily basis on farm and by veterinary surgeons who attended for injections.

Table 2 Foot disorders and other problems with the musculoskeletal system assessed daily and by veterinarians in control and BST-treated cows (PAMP data)

		Daily Inspection			Veterinarian Inspection		
Disorder Parity		Control	BST Treated	P if <0.05	Control	BST Treated	P if <0.05
Hock	Cows	0	1		5	14	0.013
Primiparous	**Days**	**0**	**1**		**10**	**72**	**<0.001**
Hock	Cows	1	4		20	28	
Multiparous	**Days**	**1**	**10**	**0.003**	**42**	**84**	**<0.001**
Foot	cows	25	34		18	25	
Primiparous	**Days**	**49**	**117**	**0.001**	**34**	**43**	
Foot	cows	31	68	<0.001	32	49	0.035
Multiparous	**Days**	**117**	**247**	**<0.001**	**45**	**112**	**<0.001**
Gait	cows	13	19		29	29	
Primiparous	**Days**	**26**	**40**		**77**	**76**	
Gait	cows	24	41	0.025	72	90	
Multiparous	**Days**	**123**	**83**	**0.003**	**179**	**284**	**<0.001**
All Musculo-skeletal Primiparous	Cows	29	42		39	58	0.008
	Days	**100**	**148**	**0.004**	**115**	**178**	**<0.001**
All	cows	50	88	<0.001	105	126	
Musculo-skeletal Multiparous	**Days**	**253**	**322**	**0.007**	**283**	**462**	**<0.001**

Number of cows =(primiparous) 209 control, 210 BST daily; 200 control, 203 BST veterinarian
(multiparous) 356 control, 313 BST daily; 341 control, 340 BST veterinarian.

The PAMP tables from which table 2 is extracted include many musculoskelatal disorders but most of these occurred at a very low incidence.

The daily inspection and veterinary inspection data are generally similar in direction but some conditions e.g. hock disorders were more likely to be detected during veterinary inspection. The figures for gait disorder in multiparous cows are surprising because the daily inspection and veterinary inspection data were significantly different in opposite directions and it seems

improbable that, for the daily inspection, increased foot disorders was associated with reduced gait disorders.

Foot disorders make up the majority of cases and these are of great importance in relation to the welfare of the animals. The daily inspection data showed that the number of multiparous cows with foot disorders was 2.2 times higher in BST-treated than in control cows and the number of days affected was 2.1 times higher in BST-treated cows.

7.4 Conclusions

An increased incidence of foot and leg disorders associated with the long term administration of BST has been described by several authors. In the largest scale study, the number of multiparous cows with foot disorders was increased by a factor of 2.2 and the number of days affected was increased by a factor of 2.1.

As a consequence of the nature of the different foot and leg disorders there will be pain and other suffering in these animals. Hence welfare will be seriously and adversely affected as a consequence of the BST treatment.

CHAPTER 8 PROBLEMS RELATED TO INJECTION

8.1 Analysis

Since BST is administered by injection in the form of a pellet or a suspension, there is the possibility that pain or discomfort could be caused to the animal by this action.

Pooled data from three studies conducted by Monsanto and published in the United States Food and Drugs Administration (FDA) Freedom of Information Summary (FDA, 1993: Table 41) show that one week post-injection 24% of cows (maximum of 43% in one study) had visible injection-site swellings 10-16 cm long or 1-2 cm high (category 2), while 4% (max. 8%) had swellings >16 cm long or >2 cm high or other complications (draining lesion, lameness, haematoma etc) (category 3). Only 26% had no visible swelling at this time - as was the case for 93% cows injected with placebo, indicating that it was the injectate which was responsible for the lesions rather than the physical process of injection.

Swellings tended to subside over time, e.g at week 2, the category 2 swellings in the worst case study had declined from 43% to 20%, while the category 3 swellings fell from 8% to 2%. According to the Freedom of Information Summary: "over 95% of scores were completely resolved within 5 weeks of injection". However, given that cows would normally receive BST injections on a two-weekly cycle, it is likely that any adverse effects on their welfare would increase progressively along with the increasing number of swollen sites on the body (at various stages post-BST injection).

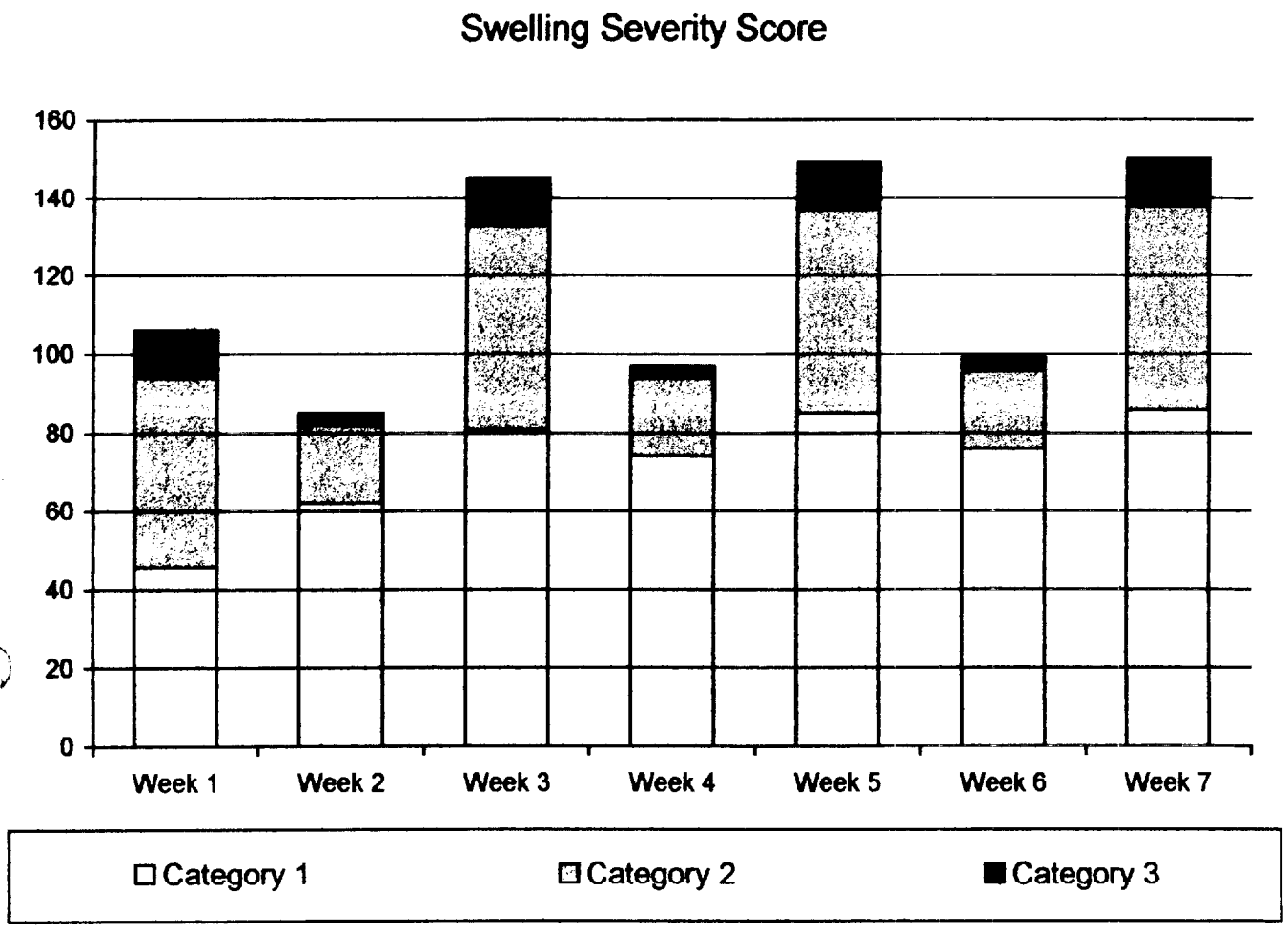

Figure 3: The 'Swelling Severity Score (SSS) attributable to injection site swellings following repeated two-weekly injections of BST (based on the data in Table 41 of the FDA 'Freedom of Information' report on Monsanto's Posilac.

Category 1: swelling<10cm long or <1cm high,

Category 2: swelling 10-16cm long or 1-2cm high,

Category 3: swelling>16cm long or >2cm high or other complications

Thus, assigning a Swelling Severity Score (SSS) of 3 to each percentage point of category 3 swellings, SSS of 2 to each % category 2 swellings and an SSS of 1 to each % category 1 swellings, it can be shown with reference to the pooled data from the three studies (see Figure 3) that the total SSS score per hundred cows following the start of a two-weekly BST injection cycle would change weekly as follows: 106, 85, 145, 97, 149, 99, 151..... Although

in the second week post-injection matters improve, as the next cycle of injections is given there is an underlying trend towards increasingly poor welfare.

In a more detailed study of a clinical field study involving 232 cows (FDA, 1993), 13 animals were selected because of persistent injection site problems. Four of these cows had injection sites scoring 2 or 3 which were at least 30 days old. Of 19 samples examined for microbial contaminants, 5 were contaminated, two with *Actinomyces pyogenes*.

Further studies were conducted on two cows that showed chronic injection site reactions (i.e. persisting for 6-12 months) and on three cows "with more typical reaction sites" (FDA, 1993). "Microscopically, granulomatous inflammation was found at nearly all sites characterised by multifocal areas containing macrophages, lymphocytes, polymorphonuclear leukocytes and giant cells. The overall reaction was supported by fibrous connective tissue while the foci of residual sometribove [i.e. the BST preparation] were apparent." By comparison with the "more typical reaction sites", in chronically reacting cows there was a "notable ... increase in the presence of polymorphonuclear leukocytes in the sites".

In what appears to be the only quantified report of injection-site lesions in a peer-reviewed scientific publication, Pell et al (1992) reported that out of 367 injections of 23 cows, ten days post-injection 10.1% cows had 'severe' (i.e. category 3) lesions, while 49.9% had 'moderate' (i.e. category 2) lesions. Out of 358 placebo injections of 23 cows in the same group, none had severe reactions and 0.6% had moderate reactions. It is clear that the BST or another component of the preparation, excluding the vehicle, is causing the problem.

According to the FDA Two Year Report on BST, between February 1994 and February 1996 there were 156 reports relating to injection-site reactions in cows treated with BST, from which the estimated percentage of cows with this clinical manifestation was 0.1% (CVM, 1996) As noted above, farmers are not likely to report such problems and any reports may refer to a proportion of category 3 swellings only. Whatever the explanation, the number of adverse reactions implied by these data is much lower than would be anticipated from the data released in the Freedom of Information Summary (FDA, 1993) and in the report of Pell et al (1992).

The dangers of BST in causing injection-site lesions are acknowledged by the manufacturers of Posilac, who recommend in their advice to users: "use of Posilac in cows in which injection site swellings repeatedly open and drain should be discontinued". Moreover, users are warned that "injection site swellings ... may remain permanent" (Monsanto, 1994)

A potential welfare problem with the injection site recommended by manufacturers i.e. the ischiorectal fossa (the tailhead) in their submission to the CVMP (CEC, 1993) has been identified. There are dangers that such a site would not only make detection of swellings more difficult but that in an area which is frequently encrusted with faecal matter the risks of infection might be increased. It is also possible that painful swellings in this area might adversely affect the usage of the tail e.g. removing flies.

Welfare might also be adversely affected by the restraining procedures accompanying injection, quite apart from the effects of the injection itself. It is difficult to define such effects accurately because they will depend partly on legal provisions (e.g. on who is allowed to administer BST) and partly on the injection procedures on a particular farm. If, as in the USA, farmers are allowed to inject their cows, concerns must arise due to some farmers' lack of training and expertise and their inability to cope with emergencies which might ensue.

8.2 Conclusion

Injection site problems occur in most cows injected with BST, but not with placebo injections, and are exacerbated by repeated injections. In 4% of cows the swelling is severe and there are occasionally chronic infections. The pain associated with this problem has not been adequately assessed.

CHAPTER 9 EFFECTS OF BST ON REPRODUCTION PROBLEMS IN COWS.

9.1 Mechanisms and preliminary studies of BST effects

The possibility that BST treatment interferes with reproduction was already evident from the first studies on the effects of BST on milk yield in dairy cows. In their report of the effects of different doses of BST on milk yield of primiparous Holstein cows, Morbeck et al (1991) noted that although days from parturition to first detected oestrus, days open, and services per conception were not affected, days from parturition to first service increased with the dose of BST, and rate of detection of oestrus decreased. Thus there was some evidence of reduced birth weight in calves and increased incidence of multiple births (Bauman et al., 1987). In a similar way, Cole et al (1992) reported that although reproductive health generally was not affected by BST treatment, delayed conception and increased incidence of abortion might occur. They also pointed out that decreased reproductive performance was an health issue requiring further evaluation. Interference of BST treatment with ovulation and oestrus detection was confirmed by several other groups (Hemken et al, 1991; Lefebvre and Block, 1992; Stanisiewski et al, 1992). These effects were not due to the handling stress accompanying BST injection since the effects of sustained-release BST did not differ from those of daily injection of BST (Zhao et al, 1992). BST did not have significant long term effects since the reproductive problems of cows treated with BST during the first lactation did not carry over upon cessation of treatment. Cows treated with BST at the first lactation and exhibiting reproduction problems at that time had a higher pregnancy rate during the second lactation, when they were no longer treated (Esteban et al, 1994a). There was no evidence of any habituation to the effects of BST on reproduction since repetition of the BST treatment during a second lactation induced the same problems as during the first lactation (Esteban et al, 1994b).

The mechanisms of effects of BST on reproduction have been investigated in both lactating and non lactating animals. BST had no effect on pituitary functions, as assessed by plasma levels of gonadotropins (Adriaens et al, 1995). The ovary is the most likely target of the effects of BST. BST increased the number of small size antral follicles (Gong et al, 1991, 1993; Kirby et al, 1997), although negative results have also been reported (Andrade et al,

1996). In lactating dairy cows, BST increased the weight of corpora lutea and the levels of IGF-I and IGFBP in the follicular fluid (Lucy et al, 1995).The effects of BST on ovarian follicular dynamics have been confirmed by De la Sota et al (1993). These authors showed that BST-treated lactating cows developed dominant follicles that were larger and less oestrogenic than those in nonlactating cows. Examination of the response of BST-treated dairy cows to a luteolytic dose of PGF2-alpha led Kirby et al (1997) to propose that BST reduces FSH, increases the turnover of dominant follicles, and induces differences in the timing of follicular waves.

9.2 Monitoring studies

The FOI summary and Post Approval Monitoring Program in the USA provide detailed information about effects on reproduction. The significant differences listed in Table 3 refer to various sample sizes and *denotes small data set.

Table 3 Significant effects on reproduction from FOI summary and PAMP survey.

Source	Measure	Primiparous / Multiparous	Control	500mg BST	p value
FOI	Pregnancy rate	primip	90%	63%	0.002
PAMP	Pregnancy rate	multip	82 ± 2.7	73 ± 3.0	0.039
PAMP	Days open	primip	134 ± 7	150 ± 7	0.048
FOI	Gestation lengt	primip	280.4 ± 0.8	277.9 ± 0.9	0.028
FOI	Gestation lengt	multip	280.5 ± 0.8	277.4 ± 0.9	0.02
PAMP	Gestation lengt	primip	279 ± 1.3	275 ± 1.3	0.001
FOI	Multiple births	primip	2.9%	20.8%	0.016 *
FOI	Multiple births	multip	1.2%	13.6%	0.003

There is evidence that BST treatment can adversely affect reproduction. Pregnancy rate dropped by 7-9% in multiparous cows and by up to 27% in primiparous cows, gestation length was shortened by 2-4 days, the number of days open increased in primiparous cows and the frequency of multiple births was substantially increased. Multiple births cause various welfare problems both for the cow and the calf. Failure to conceive by cows given appropriate opportunity, is an indicator that the cow is having difficulty in attempting to cope with the conditions in which it finds itself and hence that its welfare is poor. In the allocation of

resources within an animal, reproduction is given high priority so conditions must be stressful before conception is inhibited. Hence the measure "pregnancy rate" which indicates the proportion of animals inseminated which become pregnant reveals how many animals are so severely affected by metabolic demands and external effects on the individual that they cannot conceive. Similarly, "days open" is longer if conception is delayed so, provided that management of reproduction is adequate, a high figure for "days open" indicates poor welfare. Both of these measures showed significant differences between BST-treated and control cows. Gestation length can be shorter than normal because of the temporal advancement of parturition and this can be brought about by poor welfare. However, cows with twins usually show temporally advanced parturition and BST-treatment increased the frequency of twinning in this study so this change could have caused the changes in gestation time in the table.

Some of the effects of BST on reproduction are mediated by BST action on ovarian function via IGFI. The effects do not continue after cessation of treatment.

Concern has also been expressed about an increased incidence of retained placenta, abortion/foetal loss and cystic ovaries in BST treated animals (Canada 1999). However, more data on these possible effects are needed.

9.3 Conclusion

There is evidence that BST treatment can adversely affect reproduction. Pregnancy rate dropped from 82 to 73% in multiparous cows and from 90 to 63% in primiparous cows, gestation length was shortened by 2-4 days and the number of days open increased in primiparous cows. The effects do not carry over after cessation of treatment. The frequency of multiple births which can cause welfare problems, was substantially increased by BST. Failure to conceive is an indicator of poor welfare and multiple births lead to poor welfare.

CHAPTER 10 EFFECTS OF BST ON IMMUNOLOGY, PATHOGEN REPLICATION AND ON INFECTIOUS DISEASE IN CATTLE.

This Chapter considers experimental studies of the effects of natural Growth Hormone (GH) and BST.

10.1 Immune effects of GH

Advances in the understanding of the regulatory influences of non immune factors on immunity have revealed that in addition to its classical endocrine effects, GH is critically involved in the maintenance of lymphoid organ size and cellularity. GH receptors are present on peripheral blood monocytic cells, thymocytes and possibly lymphocytes (see for a general review Arkins et al, 1992).

10.1.1. Thymic function and hematopoiesis

GH sustains thymus growth, influences migration of T cell precursors to the thymus, and promotes the differentiation of double negative T cells (CD4- CD8-) into the double positive phenotype (CD4+ CD8+), certainly via its ability to stimulate the synthesis of thymulin from thymic epithelial cells. GH also plays an important role in the development of hematopoietic precursors and augments the in vitro maturation of erythrocytes.

10.1.2. Lymphocyte function

GH consistently augments the in vitro proliferation of lymphoid cells, possibly by acting as an autocrine factor. GH also augments a number of immune responses in vivo, including antibody synthesis and skin graft rejection, the development of adjuvant arthritis, the activity of natural killer cells, and lectin-induced T-cell proliferation and IL-2 synthesis. However, these effects are in general more easily observable in animals whose pituitary has been experimentally removed or where it is underfunctioning for pathological reasons (hypopituitary animals) than in normal animals.

10.1.3. Phagocytosis

Phagocytic cell function is influenced by GH. GH-treated macrophages acquire morphological and functional characteristics of activated macrophages. The same applies to polymorphonuclear leukocytes, and results in the enhanced synthesis of reactive oxygen intermediates (superoxyde anion). IGF-1 has similar effects on phagocytic cell functions to those of GH, including the production of oxygen reactive intermediates and tumor necrosis factor-alpha and the oxygen-dependent killing of bacteria.

10.1.4. Summary

Based on the large body of in vitro and in vivo data available concerning the effects of GH and IGF-1 on immune function, it is apparent that GH and IGF-1 are able to stimulate many components of the immune response, and specially phagocytosis. However, these effects are always more marked in hypopituitary than in normal animals, and it is important to note that the enhancement of phagocytosis that is obtained in response to GH and IGF-1 is not apparent in the absence of triggering stimuli for the activation of phagocytosis.

10.2 Immune effects of BST

Compared with what is known on the immune effects of GH and IGF-1, there have been relatively few investigations of the effects of BST administration on cattle immunity, and most of these investigations have been carried out by only two research groups, one in Canada and one in Belgium. They have used repeated daily injections of BST.

10.2.1 Haematopoiesis

Long term BST treatment in dairy cows induces a significant increase in the neutrophil fraction in peripheral blood, but reduces haematocrit, perhaps because of an increase in plasma volume (Burton et al, 1990, 1992).

10.2.2 Lymphocyte function

Treatment of dairy cows with BST enhances T cell proliferation response induced by concavalin A (Burton et al, 1991), and results in higher serum IgG and IgA concentrations (Burton et al, 1991). However, there is no change in the delayed type hypersensitivity response to dinitrochlorobenzene (Burton et al, 1992). In addition, BST reduces the inhibitory effect of high temperature on mitogen-induced proliferation in vitro, but it has no effect on the depressed lymphoproliferative response that occurs in lactating dairy cows submitted to heat stress nor does it alleviate the decreased migration of leukocytes to the mammary gland after chemotactic challenge (Elvinger et al, 1992).

10.2.3 Phagocytosis

BST treatment stimulates polymorphonuclear leukocyte (PMN) adhesiveness and release of oxygen reactive intermediates in PMN from milk and peripheral blood (reviewed in Arkins et al, 1992). These results indicate that PMN bacterial killing can be enhanced in vivo, which may result in increased resistance to mastitis. BST treatment has mixed effects on resistance to experimental models of disease in cows (mastitis; metabolic disease, response to endotoxin) but in all cases, there is no evidence for a worsening of the condition in BST-treated animals compared to controls. In an experimental model of coliform mastitis, Vandeputte-van Messom and Burvenich (1993) showed that pretreatment with BST normalises milk production and composition, but only in those animals which respond very intensively to intrammamary inoculation of *E. coli* with a respiratory burst activity of blood neutrophils. In calves injected intravenously with endotoxin, BST treatment decreased the impact of endotoxin on metabolic variables (Elsasser et al, 1996).

10.2.4 Summary of immune effects of BST

BST enhances several aspect of the immune response and tends to enhance resistance to experimental models of disease. However, the effects of BST treatment on an ongoing inflammatory response have not been assessed.

10.3 BST and viral replication

Because human recombinant GH has been reported to enhance lentivirus replication in vitro (Laurence et al, 1992), the importance of such an effect for BST and its possible adverse consequences on viral propagation within the herd have attracted attention

10.3.1 Lentiviruses

Preliminary results indicate that BST can enhance and prolong the production of Maedi Visna virus in milk macrophages of seropositive ewes and trigger the expression of caprine arthritis encephalitis virus in goat. This might be due to the presence of GH-induced transcription factors on the nucleic acid of these viruses, or to a non specific enhancement of the multiplication of virus containing epithelial cells in the mammary gland (Chilliard et al, 1998).

10.3.2 Other viruses and non conventional transmissible agents

GH alone or in combination with progesterone, oestrogens or corticoids, augments by 2 to 10 the replication of murine cytomegalovirus in vitro (Chong et Mims, 1984). GH and IGF-1 can also induce the expression of messenger RNA of PrP, the prion protein that is associated with bovine spongiform encephalopathy, in rat pheochromocytome cells in vitro. This effect, however, requires enormous doses of GH and IGF-1 that are far above those used in dairy cows.

10.4 Conclusion

The immuno-stimulatory effects of BST observed experimentally have not been confirmed clinically.

Very preliminary results indicate that GH might enhance the production of pathogenic agents that develop intracellularly, such as viruses. However, the importance of this effect for BST treatment and its functional consequences in vivo remain largely unknown.

CHAPTER 11 EFFECTS OF BST ON OTHER HEALTH PROBLEMS

11.1 Body condition

The mechanism of action of BST involves a whole range of changes in the metabolism of body tissue so that more nutrients can be used for milk production. These changes involve direct effects on tissue metabolism (e.g, adipose liver). Several papers have been published on body condition and body tissue composition. Most papers show poorer body condition in cows treated with BST. Those cows have a lower body condition score (BCS) at the end of lactation than the control animals. The difference between BC of treated and control animals varied between 0.2 and 0.5 points (FOI part 4, Wells 1995, Chilliard 1988, Phipps 1990, F01 1993). On the other hand, BST treated cows might have an increased voluntary feed intake starting 4 - 6 weeks after the onset of the treatment (FOI 1993, Oldenbroek 1990)

The body weight of a BST treated animal has been recorded as approximately 40 kg higher than control animals at the end of the lactation. However, body composition changed and this effect may be largely due to an increase in body water. (Oldenbroek, 1990; Wells, 1995; Chilliard, 1991).

11.2 Metabolic and digestive disorders

Several studies have focused on the potential adverse effect of the long-term exogenous administration of BST on health aspects of dairy cattle. Not all studies were very informative concerning study design, diagnoses etc. Conclusions such as "no health effects were noted" have been stated regularly (Phipps 1990, Hartnell, 1991, Burton 1994, Oldenbroek 1990). In general health effects are difficult to detect, because symptoms are often non specific and therefore, the prevalance and incidence of different health diagnoses, based only on visual or physical examinations are of limited value. Moreover, to study the potential adverse effect of BST on different health disorders requires large numbers of animals as most disorders occur commonly during the rising phase of lactation.

During BST treatment an increased number of cows experienced periods "off feed" (reduced

feed intake) (Monsanto 1996, Kronfeld 1994, Cole 1992, Pell 1992) There is no indication in the literature that BST-treated animals might have an increased incidence of ketosis (Burton 1994)

Several studies showed an increased incidence of bloat, indigestion and diarrhoea in BST treated cows (FOT NADA 14-872 1993, Monsanto 1996) In addition, the incidence of left displaced abomasum tended to increase BST-treated animals (Monsanto 1996). In general the control animals had more miscellaneous health problems during the pre-treatment period than the BST-treated animals. This difference might have influenced the outcome of the study (Monsanto 1996)

Several authors have described increases in laboured breathing, body temperature and heart rates in BST treated animals (Cole 1992, FOI #140-872 1993, Monsanto 1996).

One manufacturer of BST warns that udder oedema is more likely in BST-treated cows, especially when BST use is commenced in mid-lactation.

11.3 Heat Stress

The increased metabolic activity associated with BST-induced galactopoiesis also involves an increase in heat production by the body, which challenges thermoregulatory processes. The effect can be pronounced, as illustrated by the report that, of 18 cows receiving BST and subjected to heat stress, two cows died and four suffered from ataxia, whereas no such responses were observed in 16 control cows (Elvinger et al, 1992).

11.4 Culling

Concern has been expressed that cows might be metabolically overworked when treated during their lactation with BST. Therefore, life-expectation of the BST treated cows might be reduced. This effect of BST might be visible in an increased percentage of involuntary culling in herds However, the decision to cull dairy cows is complex and affected by many cow and farm factors.

Only limited information is available on culling rates associated with BST treatment. This is because of the above described reason and the fact that culling was prohibited in several of the studies.

PAMP data (1996) showed that more cows had been removed from the BST treated herds than from the control herds. The difference was significant in multiparous cows.

Ruegg et al (1998) focussed in their study on the culling practices of 32 herds. In 19 herds cows were BST treated. During the course of the study, 4 farms discontinued or restricted the use of BST and two control herds commenced BST treatment. These farms were excluded from the study. Culling rate was higher in the BST treated herds than in the control herds, although the difference was not significant. In the BST treated herds, more cows were culled because of mastitis and sickness and less cows were culled for reason of production or death, than in the control herds. A problem with this study was that the control and BST-treated herds appeared to have considerable differences in herd size, milk production levels and age at first calving.

Cole et al (1992) presented a study on health and reproduction of BST-treated dairy cows. No culling was conducted during the study and cows were only removed for scheduled necropsies or unscheduled necropsies when a cow died or was declared moribund. Eight cows had unscheduled deaths, and all these animals were BST treated. The following diagnoses were included, four mastitis cases, two pneumonias, one abomasal displacement and one case of Johnes disease.

Other studies did not reveal a high culling incidence of BST treated animals compared with control animals (Oldenbroek 1990).

11.5 Medicine usage and milk composition

BST increases the frequency of certain disease conditions such as mastitis and foot problems in cows. These conditions are normally treated using veterinary medicines. Hence BST is leading, on average to the increased use of veterinary medicines. This increased use allows more opportunity for the development of resistance to antimicrobials in pathogens on farms. It

may also result in increased residues of antibiotics in milk. These residues could result in further resistance to antimicrobials when the milk is fed to calves or other animals. This topic is the subject of another Scientific Committee report.

11.6 Conclusions

BST usage increases the incidence of several disease conditions and hence is likely to increase the usage of veterinary medicines. Increased antimicrobial usage may lead to resistance to antimicrobials with consequences for the health of humans, cattle and other animals. This topic is the subject of report of another Scientific Committee.

BST treated cows often have a lower then normal body condition at the end of lactation and experience increased "off-feed" periods

The incidence of bloat, indigestion and diarrhoea has been shown to increase in BST-treated cows.

BST lowers the ability to cope with high temperatures which in certain conditions can result in poor welfare.

The Post-Approval Monitoring Program study in the USA reported a higher culling rate in multiparous cows treated with BST.

CHAPTER 12 BST AND WELFARE: RESEARCH METHODOLOGY AND ANALYSIS

12.1 Introduction

The effects on animal welfare of all new biotechnology products used on animals, or biotechnology procedures involving genetic modification of animals, should be properly studied. In 1991 the E.U. Scientific Veterinary Committee pointed out that comprehensive studies of the welfare of cattle treated with BST had not been carried out. Some studies have now been carried out and the conclusions stated in this report have been reached but wide ranging studies of animal welfare are still needed.

A problem with published research on BST is that many studies were made only on animals injected with BST for one or two lactations. The long-term effects of BST usage are not adequately known and there could be exacerbation of the effects discovered so far, or new effects. Other problems with published research are summarised in section 6.4.1

12.2. Interpretation of data linking BST, welfare and milk yield

Poor welfare such as that associated with mastitis, foot or leg problems, some reproductive disorders or other production-related diseases can be caused by high milk yields (see Chapter 3). BST increases milk yield and also thereby increases these problems. The problems in interpretation of BST effects which this raises will now be discussed.

Results of meta-analyses, including those of Willeberg (1993), White et al. (1994), FOI (1996), Monsanto (1996) and Health Canada (1999) show that there is a significant excess risk of mastitis in the BST-treated group over the non-treated group during the treatment period equivalent to 15 - 79%, when the BST effect is estimated across individual studies. Similarly, foot disorders can be doubled and the proportion of cows which fail to conceive increased by 50-70% in BST treated cows. Some or most of these effects might be expected as a consequence of increased milk yield.

White et al. (1994), used logistic regression analysis to examine the effect of BST treatment on the risk of clinical mastitis, while milk yield, parity and study were included as co-variates. There was a significant linear relationship between milk yield and clinical mastitis during treatment, and when the increase in milk production was controlled for, the BST effect

became statistically insignificant. No parameter estimates of the effects, however, were provided. These results were used by White et al. (1994) to argue that the effect of BST on clinical mastitis is due to an indirect causal effect mediated through the increase in milk yield:

BST ——> milk yield increase ——> clinical mastitis increase

This was taken as evidence for no harmful effect of BST as such on the occurrence of clinical mastitis. The argument has also been presented that other milk production enhancing factors, e.g. genetic improvement, will have similar mastitis etc. increasing properties, but such measures are not being similarly investigated and questioned. It has been argued that, if instead of using BST one could genetically increase the milk yield by the same amount, the number of clinical mastitis, foot disorder and reproductive disorder cases would increase similarly without any official concern.

The indirect effect has been quoted by some as the ultimate explanation and the main reason for accepting that the issue of mastitis etc. has been resolved (CVMP 1993). However, the FDA has not accepted this argument and therefore such analysis has not been introduced among the FOI and PAMP analyses or in the Health Canada analyses. The study by White et al. (1994) showed that BST increases milk yield which increases the risk of clinical mastitis. It should be noted, therefore, that the analysis which included milk yield as a co-variate violated the basic epidemiological rule, that an intermediate variable in a causal pathway should never be considered as a confounder and should therefore not be introduced as a co-variate in a multivariate analysis (see e.g. Greenland & Neutra 1980, Weinberg 1993, Joffe & Greenland 1994). Kleinbaum et al. (1982) wrote :" A pure intervening variable (B in: A ‡ B ‡ C) should not be considered as a potential confounder, since its control can spuriously reduce or eliminate any manifestation in the data of a true association between exposure (A) and disease (C)". The rationale behind this is, that epidemiology has the practical purpose of discovering relations which offer possibilities of disease prevention and for this purpose a causal association may be defined as an association between categories of events or characteristics in which an alteration in the frequency or quality of one category is followed by a change in the other (MacMahon & Pugh 1970). If one wants to make sound epidemiological estimation of the causal effects of an exposure, it is therefore wrong to try to distinguish or separate indirect from direct effects - they both count in estimating the disease promoting effect of exposure to the primary variable in question (BST). Therefore, the combined effect is the best estimate of that caused by introducing BST and similarly of the preventive effect of abolishing BST treatment once it may have been introduced. Accordingly, the total effect of BST is the only meaningful parameter and this effect is unbiasedly estimated only by the risk difference

(attributable risk), which in the study of White et al. (1994) amounts to 8.3 cases of clinical mastitis per 100 BST treated cows, equivalent to 42 % above the risk in non-treated cows.

A proper and critical epidemiological evaluation of the indirect effect argument thus results in the conclusion, that such analysis and the conclusions drawn from it have confused the issue, not resolved it.

Two further, very significant flaws in the argument that increases in mastitis, foot disorders, reproductive problems etc. are acceptable because they are just a consequence of increased milk yield are: (i) that the poorer welfare would not occur in these animals if the BST were not used and (ii) that BST usage often results in such poor welfare, associated with serious mastitis, foot disorders and some reproductive problems, that there is severe and unnecessary pain, suffering and distress. Methods of dairy cow management which have such avoidable effects are not acceptable. The cow which has severe, clinical mastitis suffers, irrespective of whether or not the causal factors are direct or indirect (Willeberg 1994).

The relationships between BST use, milk yield and production related welfare problems such as mastitis, foot disorders and reproductive disorders are as follows. 1. An increase in milk yield leads to a steepening increase in mastitis etc. as the upper end of the range of possible milk yields is approached. 2. BST increases the milk yield and hence causes a small effect on the risk of mastitis etc. in low producing cows but an increasingly large effect on mastitis etc. as the pre BST treatment yield increases high producing cows. 3. Most farmers use BST to make high yielding cows into very high yielding cows. 4. Hence BST causes a substantial increase in the risk of mastitis etc. on most farms and this risk, with associated poor welfare, would not occur if BST were not used.

12.3 Management factors and the use of BST

Quality of management is a major factor determining milk yield response as is the quantity and quality of feed provided. As an example, good management measures recommended by a product manufacturer to ensure a high response in milk yield to BST administration include;

- Cows should not be overcrowded

- Additional ventilation or cooling systems may be needed if not adequate.
- Flooring should be kept clean and provide adequate traction
- Feeding areas should be designed to facilitate feeding
- Adequate water must be provided
- Cows should be protected from the effects of heat in hot weather and adequate shade should be provided.
- High quality feed should be available
- Fly control is imperative.

It is evident that such measures would improve cow welfare. However, use of BST in the absence of such measures would exacerbate welfare problems.

It has been suggested that, if there are adverse effects in cows treated with BST, the farmers are not managing their animals well enough. Hence farmers who do find that their cows have mastitis, foot disorders, reproductive disorders or other problems specified as a potential risk when bovine somatotrophin is used may be reluctant to report the occurrences. Any failure of farmers to report problems would affect the results of follow-up studies after BST use.

12.4 Conclusions

It remains to be discovered whether injection of cows with BST over the long-term, i.e. over a lifetime of lactations, will result in more severe or new effects on welfare than those reported so far.

Where BST increases milk yield and also thereby increases mastitis, foot or leg problems, reproductive disorders or other production-related disease, then BST is causing poor welfare which would not occur if it were not used. The conclusion which should be drawn is that avoidable actions which result in poor welfare, such as BST usage, should not be permitted.

It has been suggested that, if there are adverse effects in cows treated with BST, the farmers are not managing their animals well enough. As a consequence, adverse effects are likely to be under-reported by farmers.

CHAPTER 13 CONCLUSIONS AND RECOMMENDATION

The Conclusions to this report have been grouped into four sections:

- The welfare of high yielding dairy cows.
- The use of BST, the mechanisms of BST action in cows and effects of BST which do not necessarily affect the welfare of cows.
- The scientific quality of conclusions reached in papers which might seem relevant to cow welfare or which are about possible effects on cow welfare that appear not to have been investigated.
- Animal welfare and the effects on welfare of dairy cows when BST is used.

The welfare of high yielding dairy cows.

1. There is already evidence of welfare problems in dairy cows, for instance more than 50 cases of foot disorders and more than 40 cases of mastitis per 100 dairy cows can typically occur in Europe per year. Some of these animals and others in the herd may have reproductive disorders and other production related diseases.

2. There is clear evidence from several countries of significant positive associations between milk yield and mastitis, foot disorders, reproductive disorders and other production related diseases.

The use of BST, the mechanisms of BST action in cows and effects of BST which do not necessarily affect the welfare of cows.

3. Commercially produced BST is very similar in structure to naturally occurring BST. It is recommended by one manufacturer that dairy cows should be given an injection of BST once every 14 days.

4. It has been suggested that, if there are adverse effects in cows treated with BST, the farmers are not managing their animals well enough. As a consequence, adverse effects are likely to be under-reported by farmers.

5. The primary galactopoietic effect of BST in cows appears to be altered nutrient utilisation and mobilisation of non-mammary tissues, sparing nutrients for milk synthesis.

This is achieved by effects on liver and adipose tissue but also by alterations in the responsiveness of other tissues to metabolic hormones.

6. BST increases cardiac output and heart rate and this is associated with an increase in the rate of mammary blood flow. Mammary metabolic activity is increased, involving greater substrate uptake and synthesis of milk-specific components. IGF1 seems to be largely responsible for such effects. In consequence, when BST is used, milk yields increase by about 10%, with compositional changes depending on the cow's energy status, IGF1 increases approximately five fold in cow's milk.

7. Based on the large body of in vitro and in vivo data available concerning the effects of GH and IGF-1 on immune function, it is apparent that GH and IGF-1 are able to stimulate many components of the immune response, and specially phagocytosis. However, these effects are always more marked in hypopituitary than in normal animals, and it is important to note that the enhancement of phagocytosis that is obtained in response to GH and IGF-1 is not apparent in the absence of triggering stimuli for the activation of phagocytosis.

8. It appears that BST extends the period of metabolic stress which normally accompanies the onset of lactation. The cow remains in negative energy balance, utilising food reserves or other tissues, for some weeks after the commencement of BST usage.

9. The consequences of BST, acting as a neuropeptide, on the brain and on behaviour are not known.

The scientific quality of conclusions reached in papers which might seem relevant to cow welfare or which are about possible effects on cow welfare that appear not to have been investigated.

10. It has been stated in certain published papers that BST has no effects on some welfare measures e.g. mastitis, foot disorders, health in general, or welfare in general. However, these are misleading statements because the sample sizes used were too small to justify such conclusions.

11. Questions about the effects of elevated IGF1 levels in the cow on the welfare of the cow, or the welfare of the calf in utero, appear not to have been investigated. Neither have questions about the effects of elevated IGF1 levels in milk on the welfare of calves which drink the milk.

gestation length was shortened by 2-4 days and the number of days open increased in primiparous cows. The effects do not carry over after cessation of treatment. The frequency of multiple births which can cause welfare problems, was substantially increased by BST. Failure to conceive is an indicator of poor welfare and multiple births lead to poor welfare.

20. The immuno-stimulatory effects of BST observed experimentally have not been confirmed clinically.

21. Very preliminary results indicate that GH might enhance the production of pathogenic agents that develop intracellularly, such as viruses. However, the importance of this effect for BST treatment and its functional consequences in vivo remain largely unknown.

22. BST treated cows often have a lower then normal body condition at the end of lactation and experience increased "off-feed" periods

23. The incidence of bloat, indigestion and diarrhoea has been shown to increase in BST-treated cows.

24. BST lowers the ability to cope with high temperatures which in certain conditions can result in poor welfare.

25. The Post-Approval Monitoring Program study in the USA reported a higher culling rate in multiparous cows treated with BST.

26. BST usage increases the incidence of several disease conditions and hence is likely to increase the usage of veterinary medicines. Increased antimicrobial usage may lead to resistance to antimicrobials with consequences for the health of humans, cattle and other animals. This topic is the subject of a report of another Scientific Committee.

General conclusion

BST is used to increase milk yield, often in already high-producing cows. BST administration causes substantially and very significantly poorer welfare because of increased foot disorders, mastitis, reproductive disorders and other production related diseases. These are problems which would not occur if BST were not used and often results in unnecessary pain, suffering and distress. If milk yields were achieved by other means which resulted in the health disorders and other welfare problems described above, these means would not be acceptable. The injection of BST and its repetition every 14 days also causes localised swellings which are likely to result in discomfort and hence some poor welfare.

Recommendation

BST use causes a substantial increase in levels of foot problems and mastitis and leads to injection site reactions in dairy cows. These conditions, especially the first two, are painful and debilitating, leading to significantly poorer welfare in the treated animals. Therefore from the point of view of animal welfare, including health, the Scientific Committee on Animal Health and Animal Welfare is of the opinion that BST should not be used in dairy cows.

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Acknowledgements

This report of the Scientific Committee on Animal Health and Animal Welfare is substantially based on the work of a working group of the Committee.

The working group was chaired by Prof. D. Broom. The members of the group were as follows; Prof. D. Broom, Dr. R. Dantzer, Prof. P. Willeberg, Prof. B. Mepham, Prof. E. Noordhuizen-Stassen.

EUROPEAN COMMISSION
DIRECTORATE-GENERAL XXIV
CONSUMER POLICY AND CONSUMER HEALTH PROTECTION
Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

XXIV/B3/SC4/32 final

Report of the

SCIENTIFIC COMMITTEE ON VETERINARY MEASURES

RELATING TO PUBLIC HEALTH (SCVPH)

on

Public Health Aspects of the Use of Bovine Somatotrophin

15-16 March 1999

INDEX

INTRODUCTION

Mandate

The Scientific Committee on Veterinary Measures relating to Public Health is asked examine the use of bovine somatotrophin (BST) to dairy cows as a productivity aid to milk production. In particular the Committee is invited to assess the possible direct and indirect adverse effects on public health caused by the use of BST under normal conditions.

In a parallel exercise, the Scientific Committee on Animal Health and Animal welfare is asked to report on the incidence of mastitis and other disorders in dairy cows and on other aspects of the welfare of dairy cows.

Background

Council Decision 94/936/EC of 20 December 1994 amending Decision 90/218/EEC concerning the placing on the market and administration of bovine somatotrophin (BST) prohibited the marketing and the use of BST in the EU until 31 December 1999.

The Council asked the Commission to entrust a Working Party of independent scientists with the task of assessing the effects of using BST, in particular as regards the impact of the use of this product on the incidence of mastitis. In this request it is stated that "BST is an issue which gives rise to considerable interest among consumer, agricultural and industry interests. In this context, concerns have been expressed about the safety to humans, animals and the environment, the quality of milk, the economic and social consequences in agriculture, the climate for research and development, industrial competitiveness and trade implications".

Comment

The present report is limited to the public health aspects. The abbreviation BST is generally used to indicate recombinant bovine somatotrophin (rBST).

EFFECTS OF rBST ON PUBLIC HEALTH

Products containing or consisting of rBST are veterinary medicinal products within the meaning of Directive 81/851/EEC on the approximation of the laws of the Member States relating to veterinary medicinal products. In the case of veterinary products derived from biotechnology, Community concertation procedures established by Directive 87/22/EEC have to be taken into account as well, implying that the advice of the Committee for Veterinary Medicinal Products (CVMP) must be obtained before any decision on the authorisation of individual products can be accepted. Recombinantly derived BST products (rBSTs) may have slightly different chemical structures from natural BST produced by the pituitary gland, by adding a number of amino acids. Thus, each product must be considered on its own merits by the CVMP and it should be emphasised that it is not the aim of this report to provide an expert opinion on certain veterinary medicinal products.

In drawing up this report, the working group has made use of previously compiled reports by regulatory and advisory authorities in which aspects of the safety, quality and efficacy of rBST products has been examined. In particular, reference is made to:

(1) 1st report concerning Bovine Somatotrophin (BST) COM89, 379 final

(2) 2nd report from the Commission tot the Council and to the Parliament concerning Bovine Somatotrophin (BST) SEC(91) 2521 final (16.01.1992)

(3) CVMP-European Commission, DOCs. No. III /3006-7/93, 23 January, 1993

(4) FAO FNP 41/5: Food and Nutrition paper: Residues of some veterinary drugs in animals and foods. Bovine Somatotropins (1993)

(5) Communication from the Commission to the Council concerning Bovine Somatotrophin (BST) update SEC(94), 1713 (25.10.1994)

as well as the recent

(6) Report of the JOINT FAO/WHO Expert Committee on Food Additives, presented at the 50th meeting in Rome, 17/26.02.1998, (WHO: Food Additive Series 41, pp. 125-146, 1998)

(7) Health Canada Report on BST (1999)

(8) Ongoing discussions in Codex Alimentarius

In addition, recent scientific literature, in particularly those which became available after 1994 have been considered, as indicated in this report (references section A). Finally a number of reports and opinion statements have been considered as summarised in section B of the references.

1. THE RATIONALE OF RISK ASSESSMENT AND RISK MANAGEMENT IN THE CONTEXT OF PUBLIC HEALTH

Risk assessment and risk management are not only scientific and technical activities, but also represent a task attributed to science from the society. In principle, risk assessment should represent a formally defined and socially accepted evaluation process, which is separate and independent from the decisions concerning risk reduction or risk elimination. This separation and independence was considered appropriate for preventing possible biases in the risk assessment process, which could be caused by influencing the desired neutrality of the evaluation. Based on this principle, risk assessment should be a matter of scientific evaluation, whilst risk management should be a matter of political and social decision making. Thus, additionally to the scientific procedure of risk assessment the following issues may be considered in risk management:

The perception of involuntary risk factors (consumer's expectations and concerns).

The uneven distribution of risk and benefits (e.g. health and/or economic advantage).

Risk assessment should cover the following items:

(1) Hazard identification

(2) Hazard characterisation: dose (concentration) – response (effect) assessment

(3) Exposure assessment

(4) Risk characterisation

As far as risk assessment is concerned, the following definitions are applied:

Hazard identification

Identification of the adverse health effects related to the intrinsic properties of a substance.

Hazard characterisation

Qualitative and/or quantitative evaluation of the nature of the adverse health effects. This implies a dose (concentration) – response (effects) assessment and an estimation of the relationship between dose (or level of exposure) to a substance and the incidence of a biological effect (response).

Exposure assessment

Qualitative and/or quantitative estimation of the concentrations/doses to which human populations (here: consumers) are exposed. Exposure assessment requires information about the effects of production, processing, handling, and consumption of respective food commodities.

Risk characterisation

Estimation of the incidence and severity of the adverse effects likely to occur to a human population. Thus, the risk characterisation should "*include a qualitative and/or quantitative estimation, including attendant uncertainties of the probability of occurrence and severity of known or potential adverse health effects*" as specified in a document on "Risk Assessment; Towards internationally acceptable standards for food additives and contaminants bases on the use of risk analysis" (Hugett et al., (1998).

In conclusion, risk assessment can be regarded as scientific essentiality directed to provide suitable answers to two questions:

(1) What is the probability or likelihood of an undesired event to occur, and

(2) What are the consequences of this undesired event in qualitative and quantitative terms.

Thus, by definition, basic risk assessment excludes in its initial phase concerns of the decision making bodies and neglects the comparison and balance of risk and benefits and societal requests related to ethical, economic, technical and political aspects (EU, 1996, Technical Guidance Document in Support of the Council Directive 93/67/EEC on Risk Assessment of New Notified Substances and Council Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances).

2. PUBLIC HEALTH ASPECT IN TERMS OF SAFETY OF MILK AND MILK PRODUCTS DERIVED FROM RBST TREATED LACTATING COWS

2.1. Hazard identification

2.1.1. BST and its metabolites

Growth hormone (GH, somatotrophin ST) belongs to the protein family of somatolactogenic hormones. In the 1980s advances in recombinant DNA techniques made sufficient quantities of recombinant bovine growth hormone (rBST) available for the use as milk production enhancing agent. No therapeutic applications of rBST have emerged in veterinary medicine (Burton et al., 1994).

The application of rBST to dairy cows involves a parenteral application due to the instability of BST in the gastrointestinal tract. Following application and based on the peptide nature of rBST, rapid degradation by cytosolic proteases and lysosomal enzymes which are virtually present in all cells takes place. Residual amounts of rBST may be expected at the site of injection and in muscle and connective tissues especially following improper administration of rBST formulations. The major identified metabolite of rBST in plasma was the same as the physiological thrombin cleavage product of BST. This was demonstrated by sequence analyses in which two fragments were found in a close to equimolar ratio. One sequence was homologous to the N-terminus of the BST protein, whilst the other sequence represented a fragment produced by cleavage at the same site as the thrombin cleavage site of the BST molecule (Bang et al., 1994b, Bang, 1995, Bang and Fielder, 1997). No formal risk assessment has been applied to these cleavage products.

Hence there is no evidence that intact BST or one of the above mentioned cleavage products exert any direct biological effect after oral ingestion in humans and in consideration of the heat-lability of rBST during pasteurisation, non-specified ADI- and MRL values have been considered for rBST (FAO/WHO Expert Committee on Food Additives, Rome, 1998).

2.1.2. IGFs

Elevated levels of pituitary growth hormone are associated with increased liver secretion of IGF-I and its binding proteins and chronic inhibitory control of GH secretion is mediated by IGF-I feeding back to all upper levels of the GH regulatory pathway.

Thus, in particular long-term metabolic effects of GH or its analogues (rBST) are considered to reflect the regulation of expression of certain genes. GH regulated genes in the liver include the gene encoding for IGF-I and recent work indicated that other tissues including adipocytes and chondrocytes increase IGF-I mRNA expression in response to GH. IGF-I has a high affinity for a family of IGF-binding proteins, which modulate its biological actions. Regulation by GH of these genes encoding for binding proteins is considered as another relationship between GH and IGF-I. In addition, several other genes have found to be regulated by GH including the *spi*2.1. gene, encoding a liver specific serine protease inhibitor and the genes encoding cytochrome P450 enzymes (particularly the CYP2C family, see also section 3) responsible for the biotransformation of numerous pharmaceuticals and other xenobiotics (for review see Carter-Su et al., 1996).

As the increase of circulating IGF-I under the control of GH is considered as one of the physiological mechanisms of GH, the application of rBST is expected to induce the same mechanism. Indeed following the zootechnical application of BST an increase in circulating IGF-I concentrations has been found in lactating dairy cows (for details see section 2.3.). Hence IGF's are single chain polypeptides, they are excreted into milk. This has been confirmed in different animal species including humans. The amino acid sequence of IGFs is highly conserved in mammals, and bovine and porcine IGF-I are identical to human IGF-I (Honegger and Humbel, 1986, Francis et al., 1989a,b), while IGF-II sequences exhibit a greater variation among different animal species.

IGFs possess endocrine, paracrine and autocrine activities. IGF-I acts as a progression factor in the cell cycle and has mitogenic and anti-apoptotic properties. IGFs are involved in numerous physiological cell differentiation processes embodying for example cellular differentiation in perinatal development as well as processes such as maturation of ovary cells and regular apoptosis, and cell proliferation. The numerous medical reports (more than 1000 per year in the last two years) focus on both aspects, the possibility of the use of IGF-I in the treatment of distinct diseases, among others insulin independent diabetes and renal failure, whilst others describe the detrimental role of IGF-I as cellular growth regulator and tumour promoter. The plethora of biological effects exerted by IGF-I in vitro needs to be translated to the complexity of mechanisms in the intact organism before a final evaluation of dose-dependent effects can be made.

2.1.3. Additional hazards

In identifying the potential hazards, secondary risks related to the use of rBST in dairy cows need to be considered as well. These arise from possible changes in milk composition of treated animals and impairment of animal health, in particular the increased incidence of mastitis resulting in a more frequent use of antimicrobial substances (as discussed in more detail in the report on the animal welfare aspects).

2.2. Hazard characterisation: Qualitative and quantitative evaluation of the nature of intrinsic biological properties of IGFs

As it has been mentioned above, during the last five years, an explosion of new information has confirmed and extended the understanding of the pleiotropic effects of the IGF system on growth, development, and intermediary metabolism (Stewart and Rotwein, 1996). The insulin-like growth factors (IGFs) comprise a conserved pair of secreted proteins, **IGF-I** (previously termed somatomedin C) and **IGF-II** (termed somatomedin A). IGF-I is a single-chain basic protein of 70 amino acids, and IGF-II is a slightly acidic single-chain peptide of 67 residues (Rinderknecht and Humbel, 1978a,b). By molecular cloning it could be demonstrated that both IGFs are highly conserved proteins found in an array of vertebrate species (for recent reviews, see Rotwein, 1991, Dugay et al., 1995).

Circulating IGFs are bound to carrier proteins, denoted **IGF bindings proteins** (IGFBPs). It soon became evident that IGFBPs comprises a family of at last six members, and a diversity of functions has been attributed to these proteins, which prolong the half-life of circulating IGFs, facilitate the transport of IGFs from the circulation to the peripheral tissues, and thus potentiate or inhibit IGF action (Bach et al., 1994; Jones and Clemmons, 1995; Chan and Spencer, 1997; Hossner et al., 1997; Lee and Giudice, 1997)

The cellular effects of IGFs are mediated by two distinct receptors. The **IGF-I receptor (IGF-IR)** is a hetero-tetrameric glycoprotein which may be produced by mRNAs derived from a single 21-exon IGF-IR gene, located on chromosome 15q25-q26 although several receptor variants have been described (Abbott et al., 1992). The IGF-IR is similar in topography and sequence to the insulin receptor and shares >50% amino acid identity (Ullrich et al., 1986). The receptor is composed of two ligand binding α-subunits and two transmembrane β-subunits. Ligand binding to the α-subunit triggers activation of the intracellular tyrosine kinase, leading to receptor autophosphorylation by an intra-molecular trans-mechanism similar to that used by other receptor-tyrosine kinases (Leroith et al., 1995).

Functional analysis of IGF-IR revealed a complex signal transduction pathway as activation of the IGF-IR by ligand binding causes not only rapid tyrosine phosphorylation but also the intracytoplasmatic assembly of a complex consisting of a variety of proteins (SH2-containing proteins including Grb2, GAP, SH-PTP2, p85, Nck and Sc), which link this receptor to the stimulation of the protooncogene p21 *ras* and the mitogen-activated protein (MAP) kinase pathway and thus overall regulation of gene expression (Davis, 1994). Activation of phosphatidylinositol-3-kinase via IGF-I signalling pathways leads to the induction of several biological effects, including stimulation of hormone-sensitive glucose transport (Cheatham and Kahn, 1995) and activation of the enzyme p70S6 kinase, which may be involved in mitogenesis (Cheatham et al., 1995; Baserga, 1995).

Over-expression of human IGF-IRs in mouse and rat fibroblasts has been found to induce neoplastic transformation and development of tumours when transfected cells were introduced into immunodeficient nude mice (Kaleko et al., 1990). These findings indicate the potential role for IGF-IR in tumour genesis (see below).

Finally IGF-IR is involved in the signalling pathway of other growth factors including epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) (Coppola et al., 1994; Deangelis et al., 1995) and at least two dominant oncogenes (large T antigen of *simian virus 40* and the *ras* and SRC oncogenes and tumour suppress genes (Sell et a., 1993, Valentis et al., 1994, Sell et al., 1994; Werner and Leroith 1995, Neuberg et al., 1997).

The IGF-II receptor (IGF-IIR) is a single-chain membrane-spanning glycoprotein that also is known as cation-independent mannose-6-phosphate receptor. The IGF-IIR is highly conserved among different species, with ~80% identity being found among bovine, rat, mouse, and human receptors (Kornfeld, 1992). The IGF-IIR is uniquely involved in the clearance of lysosomal enzymes from the extra-cellular environment. For example, the receptor plays a role in the uptake of thyroglobulin after its secretion by thyroid follicular cells and its subsequent degradation in lysosomes (Herzog et al., 1987). It has been shown that IGF-IIR binds the latent form of transforming growth factor-β1 (TGF-β1) and that this binding seems to be essential for growth factor activation pointing to the role of IGF-II in fetal development (Korner et al.., 1995, Lau et al., 1994).

However, genetic studies have not depicted a signalling function for the IGF-IIR and thus the role for the receptor in mediating IGF-II actions remains to be substantiated (Flaumenhaft et al, 1993, Korner et al., 1995).

The physiological actions of IGF-I and IGF-II relate to growth and development of the embryo and fetus and to cellular differentiation, proliferation and cancer.

Over-expression of bovine, murine or rat GH causes increased growth in transgenic mice accompanied by two- to threefold elevations in serum IGF-I concentrations (Mathews et al., 1988a). Transgenic mice expressing human IGF-I in the liver and other tissues also showed enhanced growth (Mathews et al., 1988b), while mice over-expressing IGF-II did not (Wolf et al., 1994). Over-expression and subsequent increase of serum IGF-I levels manifest as selective organomegaly rather than increase in skeletal size. This indicates that the effects of GH or IGF-I on rate of growth on individual organs and in the entire animal are not identical. IGF-I stimulates a greater increase in kidney, spleen and thymus weight than GH (Skottner et al., 1989). These qualitatively different responses to GH and IGF-I might be related to the fact that GH induces IGF-I synthesis in multiple tissues and also enhances the expression of the major serum carrier protein IGFBP-3 and its cofactor ALS (acid labile subunit) in liver. The consequence of the induction of the expression of this ternary complex is a more sustained exposure of all tissues to IGF-I. IGF-I can stimulate the expression of IGFBP-3 but has no effect on ALS synthesis.

Depending on the study, mice with a disrupted IGF-I gene were significantly smaller in weight and length than wild-type litter mates (Powell-Braxton et al., 1993). Although this confirms that IGF-I and IGF-IR are necessary for normal embryonic and fetal growth, IGF-II seems to be essential. Despite numerous reports on IGF-II gene expression and its regulation by parenteral imprinting in rodents, comparable information from humans is scarce. However, a concordant loss of imprinting of the human IGF-IIR gene promoters has been found in certain cancers (Zhan et al., 1995).

In conclusion, the results described above, in conjunction with other known growth factor signalling pathways and oncogene-mediated cell transformation, provide the evidence for the role of IGFs in tumorigenesis (Yang et al., 1993; Sell et al., 1995, Minniti et al., 1995). However, when critically examining this information it has to be concluded that IGF action is involved in multiple biological processes thus rejecting the possibility to define a dose-effect relationship which describes all individual events.

2.3. Exposure assessment: Occurrence and detection of BST, rBST and IGF-I

Exposure assessment of food contaminants comprises direct measurements indicating the presence and quantity of the compound under investigation in certain food commodities and molecular epidemiology providing evidence of past exposure based on the analysis of typical biomarkers (for example DNA- or protein adducts), or selected somatic cell mutations, if appropriate.

Exposure assessment as applied to chemically defined feed supplements or veterinary medicinal products, e.g. compounds which are used on purpose (intentionally) in food production processes comprises the evaluation of the fate of the compound in the target animal species (distribution and disposition of the parent compound and its biological active metabolites) with the aim to describe the time dependent (target animal) body clearance and thus the quantity and likelihood of the occurrence of residual amounts of the parent compound or its biologically active metabolites in edible tissues, milk and eggs.

rBST closely resembles the physiologically expressed, endogenous bovine growth hormone and is designed to exert the same effects as this natural hormone in dairy cows. Thus, provided that rBST is used in animal husbandry, two general questions need to be addressed:

(1) What is the state of art in analytical methodology for the discrimination between endogenous growth hormone profiles and zootechnically applied rBST?

(2) What is current knowledge on the occurrence of residual amounts of rBST remaining at the injections site and to what extend secondary, biologically active metabolites such as IGFs are detectable in edible tissues and milk as a consequence of rBST treatment.

2.3.1. Analytical methodology: State of the art in the discrimination between non-treated and rBST-treated cows

2.3.1.1. GH and BST

Formerly, the analytical methods used to determine GH (bovine growth hormone; bovine somatotrophin (bST)) concentrations in plasma, milk and tissue of cows were exclusively radio-immunoassay procedures. None of them were able to distinguish between the endogenous bST and the recombinant growth hormone (rBST) products.

However, this assay was applied to compare bST and IGF-I levels in tissues of control animals and rBST treated animals (Choi et al., 1997). Although a tendency towards a dose-related increase of tissue (muscle) was observed, the differences between control animals and rBST treated animals were statistically not significant.

Since 1990 a number of interesting developments have been launched. Electro-spray mass spectrometry has been used to determine the differences in molecular mass between the natural bST and one of the recombinant products (Somagrebove®). Purified preparations of bovine pituitary bST and rBST were used (Scippo et al., 1997) and the accuracy of the technique was proven to be about 0.05 % of the mass of the protein. This corresponds to 11 Dalton for a protein of about 22000 Dalton, which is more than enough to detect a difference of one amino acid, as the average molecular mass of an amino acid is 115 Dalton. For rBST (Somagrebove®) a molecular weight of 22103 Dalton was measured, whereas the theoretical molecular mass is 22094 Dalton. For the natural bST the mass spectrum is much more complicated because theoretically four variants exist. These have either 190 or 191 amino acids (phenylalanine or alanine-phenylalanine at the N-terminal) with a heterogenecity at position 127 (valine or leucine). The two most dominant variants (190 and 191 amino acids with leucine at position 127) give peaks corresponding to molecular masses of 21725 Dalton and 21796 Dalton respectively, whereas the theoretical values are 21720 Dalton and 21791 Dalton. For the detection of rBST treated cows, the authors suggest to apply this technique on milk and plasma samples after purification and concentration by immuno-affinity chromatography. The minimum amount needed to be obtained by this concentration steps is approximately 5 to 10 pmoles, which corresponds to 0.1 to 0.2 µg. Considering that the minimal concentration of bST in plasma is in the range of 1 ng/mL in non-treated cows, this means that approximately a 100 mL plasma sample will be required for analytical procedures as described.

Several attempts have been made to measure bST concentrations in milk or in plasma by non-radioisotopic immunoassays. A biotin-avidin sandwich enzyme-linked immunosorbent assay for the determination of bovine growth hormone in plasma has been developed by Secci et al. (1988). Affinity-purified antibodies are immobilised on microtiter plates. Bovine GH bound to the specific antibody is then detected with a second anti-bovine GH antibody labelled with biotin and peroxidase-conjugated avidin. This method

has a sensitivity as low as 0.25 ng/mL plasma. No applications for the detection of rBST administration are reported as of yet.

An avidin/biotin ELISA assay for bovine somatotrophin is described by Zwickl et al. (1990). The method uses affinity-purified polyclonal antisera raised in rabbits to immobilize bST from blood or milk samples on the wells of microtiter plates. Bound bST is quantitated by adding biotinylated anti-bST antibody during the sample incubation step, followed by incubations with horseradish peroxidase labelled avidin D and ABTS substrate. Because high-affinity anti-bST antibody is used, and the biotinylated antibody is added directly to the sample, the assay can be performed in less than 4 h while sensitivities of 0.2 and 20 ng/mL in milk and blood, respectively, are obtained.

Another competitive enzyme immunoassay for bST was described by Hennies and Holtz (1993). Antiserum, raised in rabbits, is preincubated with samples and free antibodies from the reaction mixture are immobilised using a microtiter plate coated with bST. Bound antibodies remaining from the pre-incubation are visualised using a biotinylated second antibody as a bridge for subsequent amplification by an avidin-biotin-peroxidase complex. The measuring range covers concentrations between 0.5 and 100 ng/mL. A similar competitive enzyme immunoassay (EIA) for growth hormone in bovine pituitary cell culture medium has been developed by Roth et al. (1997).

Ehrard et al. (1994) developed a sandwich ELISA which is able to detect various rBSTs with different N-terminal amino acids and thus allowed the discrimination between rBST and pituitary bovine GH by an affinity factor of 2.0. The authors believe that it might be possible to identify rBST treated cows, but a field study is needed for confirmation. No results of such a field study have been reported as of yet. The other methods for the control of treated and untreated animals are all indirect methods. Various possibilities are under development.

The injection of rBST into animals gives rise to the production of antibodies against these compounds. Their presence in plasma is an indirect proof of the treatment, even after discontinuation of the treatment. A first assay measuring these specific antibodies has been elaborated (Scippo et al., 1997). ELISA plates are coated with rBST and incubated with the cows serum. In the case of antibodies present in the serum they are detected by the addition of a second antibody against bovine IgG, coupled to a peroxidase label.

2.3.1.2. IGF-I

The second type of indirect methods are related to the fact that GH and rBST application increase IGF-I levels in milk. A radio-immunoassay for IGF-I in bovine milk was developed by Zhao et al. (1991). The technique was used for the analysis of milk samples obtained from three control cows and three rBST-treated cows (41-44 weeks post partum). Mean concentrations of IGF-

I were 2.77 ± 1.36 ng/mL in control cows and 3.30±1.40 ng/mL in treated cows, respectively.

The results of IGF-I quantitative assays are controversial: the physiological reference values vary from 1 to 30 ng/mL. This variation is not only based on the reference populations of cows (inter-individual variation) but also reflects the sensitivity of the antibodies applied for the radioimmuno assays (Malven et al., 1987, Bang et al., 1994b).

In conclusion, the analytical procedures described, were designed to discriminate between treated and non-treated animals. No formal intercomparisons of the different analytical procedures are available allowing a conclusive comparison of the reported IGF-I levels in milk and dairy products.

An enzyme immuno-receptor assay for the quantitation of IGF-I and insulin receptors in bovine muscle tissue was developed by Boge et al. (1994). After solubilization with Triton X-100 receptors were immobilised in microtiter plates using receptor specific monoclonal antibodies that recognise the intracellular beta-domain of the respective receptors. The immobilised receptors were labelled with either biotinylated IGF-I or insulin. The bound ligands were detected with a streptavidin-horseradish peroxidase technique. The assay, which was fully validated, had a detection limit of 1 fmol receptor/well. The assay system was used to study the effect of growth hormone treatment upon IGF-I and insulin receptors in bovine skeletal muscle. Three groups of 12 heifers (13 months old) were treated with 320 or 640 mg rBST (slow release preparation) every fortnight for 3 months. When samples of the M.splenius were assayed for IGF-I and insulin receptors, there was no difference between groups neither with respect to receptor concentration nor affinity.

Finally, a third analytical procedure was introduced, again designed to identify rBST treated cows. This procedure is based on the fact that treatment with rBST results in a decrease of the blood levels of specific IGF binding proteins (IGFBP). The use of an immunological method (Scippo et al., 1996) allows to estimate this decrease. The concentration of IGFBP seems to be 7 times lower in treated cows compared to untreated animals. Thus, these methods would allow a reliable identification of rBST treated animals.

2.3.2. Excretion of IGF-I in milk of non-treated and treated (rBST) cows with particular reference to physiological variation during lactation

Evaluating more than 60 scientific articles covering the period 1987-1998 it can be concluded that mammalian milk contains various biological active growth factors including IGF-I peptides (for review see Xu, 1998). In bovine milk, concentrations of IGF-I have been observed in the range of:

1-34 ng/mL, normal milk (Malven et al., 1987; Campbell & Baumrucker, 1989; Juskevich and Guyer, 1990; Collier et al., 1991; Schams, 1991; Zumkeller, 1992).

100-300 ng/mL, colostrum (Francis and Read, 1986, Malven et al., 1987; Campbell & Baumrucker, 1989; Zumkeller, 1992).

4.3 ng/mL (range 1.3 – 8.1 ng/mL) average bulk tank milk prior to BST use (Collier et al., 1991)

A comparison of retail milk originating from 'labelled' milk (from non-treated cows) and 'non-labelled' milk (non-specified samples originating from treated and non-treated cows) demonstrated a small, insignificant increase of IGF-I concentrations in the non-labelled milk samples (Eppard et al., 1994). However, in this study the actual number of animals treated with commercial rBST is not known.

During a lactation period, a typical IGF-I profile in cow's milk varies from 150 ng/mL after parturition to 25 ng/mL at the end of the first week of lactation, to 1 to 5 ng/mL at day 200 of lactation (Prosser, 1988; Xu, 1998).

In 1989, the first full report on milk concentrations of IGF-I in cows treated with rBST appeared. Prosser et al (1989) showed a 3.6-fold increase in the IGF-I concentration over a 7-day period of treatment. In 1994, Burton et al. highlighted several studies demonstrating a two to fivefold increase of IGF-I as a consequence of rBST treatment (Van den Berg, 1989; Gluckman, 1990; Groenewegen et al., 1990; Juskevich and Guyer, 1990).

A broad experiment comprising daily injection and administration of a sustained release formulation of rBST, respectively, was performed with 74 lactating cows (Zhao et al., 1994). Treatments began in the fourth week of lactation and lasted 40 weeks. IGF-I was monitored through early, mid- and late lactation. rBST treatment resulted in a significant increase of plasma IGF-I in all lactation periods for both treatment groups. A higher milk IGF-I concentration, however, only occurred in mid- and late lactation periods for the daily injection group. It is worthwhile to mention that application of rBST is restricted in most cases to the mid- and late lactation.

The JECFA Report (1998) cites average control values for IGF-I in milk of 3.7 ng/mL for untreated cows, and a significant increase to an average of 5.9 ng/mL as a consequence of rBST-treatment (see FAO FNP 41/5, 1993). Similarly, studies of different pharmaceutical companies report an increase of IGF-I levels in milk between 25 and 70 percent in individual animals (Burton et al., 1994). Thus, the quantities present in the daily human consumption of milk and dairy products are much lower than the total amount of IGF-I secreted daily in the gut (saliva, gastric juice, jejunal chyme, bile, and pancreatic juice (Chaurasia et al., 1994, Bauman, 1995).

The IGF-I concentration in human breast milk at weeks 6 to 8 is 22 ng/ml (Prosser, 1988). Likewise to animals, IGF-I levels are high in the colostrum

(17-30 ng/mL) and decline during lactation period (1-10 ng/mL) (Xu, 1998 and references therein).

Milk secretions of mammals, however, also contain amino acid N-terminally truncated forms of IGF-I, which have a potency that is up to ten times greater than normal IGF-I (Francis et al., 1988; Lemmey et al., 1991). Regarding milk from cows, 3% of the IGF-I is reported to be of the N-terminally truncated form (Shimamoto et al. 1992).

Consequently, even at a 3% level, the des(3N)IGF-I contributes substantially to an increase in bioactivity.

Bovine IGF-I is not denatured by pasteurisation (79°C for 45 seconds; Miller et al., 1989). However, following processing of milk for infant formula (121°C for 5 minutes) IGF-I is no longer detectable (Collier et al., 1991). In contrast, an increase of measurable IGF-I levels up to 70% following pasteurisation have been reported as well (Juskevich and Guyer, 1990). However, the different analytical methods applied allow no direct comparison of these different reports. It is worthwhile to mention here again that bovine IGF-I has been shown to be identical in structure to human IGF-I (Honegger and Humbel, 1986; Burton et al., 1994) as mentioned before.

In conclusion, even though factors such as stage of lactation, parity, level of nutrition and age influence IGF-I levels in milk, the daily administration of rBST will increase the concentration of IGF-I in milk throughout the lactation period.

IGF-I in milk is resistant to pasteurisation and even elevated levels of IGF-I have been reported after pasteurisation. The latter might be related to the standard analytical procedures which fail to detect protein-bound (IGFBP-bound) IGF-I (see section 2.3.1.2.). Consequently, consumption of milk from rBST treated dairy cows will increase the daily intake of IGF-I.

2.4. Risk Characterisation: Bioactivity of GH and IGF-I

2.4.1. Effects of rBST and IGF-I in the Gastrointestinal Tract

Although - at least theoretically - measurable residual amounts of rBST may occur in edible tissues (including the site of application) these residues are not considered to be of public health concern as the bovine growth hormone fails to interact with human growth hormone receptors (*In contrast:* human recombinant GH is under investigation for therapeutic use in the treatment of inflammatory bowel diseases).

Thus, even persistent rBST residues in meat and milk are unlikely to be absorbed from the gastrointestinal tract and would be biologically inactive in humans. In addition, rBST in cow's milk is inactivated by pasteurisation.

In contrast, IGFs are highly conserved throughout mammalian species and bovine and human IGF-I are identical. This implies that possible biological effects of persistent and even slightly increased IGF-I levels in milk (as

discussed in section 2.2) have to be evaluated. The following questions deserve attention:

- Does the IGF-I molecule remain undestroyed in the gastrointestinal tract of humans (when products from rBST-treated animals have been consumed)?

- Based on the biological activity of IGF-I activity as cellular growth factor and assuming that IGF-I is not immediately destroyed in the gastrointestinal tract, what is the consequence of the direct exposure of the gut mucosa?

- What evidence can be provided that orally ingested IGF-I enters systemic circulation and what are the possible consequences of this systemic bioavailability?

2.4.1.1. Physiological properties and functions of IGF-I in the gastrointestinal tract

Until 1991 little attention has been focused on IGF actions in the gut (Read et al. 1991), although it had been described earlier that particularly in the fetal period the stomach contains one of the highest concentrations of IGF-I mRNA and thus the IGF-I content of the intestine exceeds that in liver. In human foetal stomach and intestine IGF immuno-reactivity is localised in epithelial cells with higher concentrations in the villus than in the crypt cells. Adult rat intestines contain slight to moderate IGF-I immuno-activity in scattered epithelial cells covering the Peyer's patches. It was concluded that gut expression of IGF-I and IGF-II is under developmental regulation. IGF-II expression was found to be maximal in foetal life declining rapidly in the early postnatal period. This pattern parallels the postnatal decline in liver IGF-II, but contrasts with the marked increase in liver IGF-I in neonatal rats.

In addition, gut tissues express several types of IGF bindings proteins including IGFBP-2 and IGFBP-3. The expression pattern differs between stomach and intestines.

Finally, IGF-I and IGF-II receptors have been identified throughout the gut of several species, including human, pig, rat and rabbit, again exhibiting tissue-specific distribution patterns. Epithelial receptor-binding activity is higher in the colon than in other parts of the gastrointestinal tract, while receptor density in the intestinal epithelium is greater in the crypts than the villi. These findings suggest that receptor expression declines with cellular differentiation (Read et al., 1991 and references therein).

Evidence that exogenous supplementation (via the intake of milk containing IGF-I) with IGF-I is essential in the postnatal phase was provided by Dvorak et al (1996). Applying a sensitive RT-PCR assay, IGF-I gene expression was measured in different age groups (rats) indicating 3 fold higher levels of IGF-I mRNA transcripts in the rat small intestine of adults than in sucklings. The authors concluded that the obvious limitation for IGF-I synthesis in suckling rats may relate to significant enteral IGF-I intake via milk.

However, exogenous IGF-I peptide as present in milk may be also responsible for the down-regulation of IGF-I mRNA expression in the developing rat gastrointestinal tract.

Of interest are also previous findings in rats and pigs indicating high postnatal concentrations of IGF-receptor specific mRNA in gastrointestinal tissues relative to the mRNA concentrations of IGF-I (and IGF-II). The temporal changes in IGF-receptor density have been found to correlate with other indicators of intestinal growth and functions (Schober et al. 1990, Burrin, 1997)

2.4.1.2. Trophic effects of exogenous IGF-I:

In animals and humans there are specific IGF-I receptors on the luminal surface of the gastrointestinal epithelium (Donovan and Odle, 1994; Zumkeller, 1992, Oguchi et al., 1997). IGF-I stimulates growth and developments of the tissue and it has been demonstrated that it increases cell proliferation in a dose-dependent manner. Investigating the rate of cell replacement in primary cultures of small intestinal epithelium, Booth et al (1995) found a dose dependent increase in epithelial growth at concentrations ranging between 0 and 20 ng IGF-I per mL.

Initial experiments by Young et al. (1990) had indicated that IGF-I administered either by oral or parenteral routes, stimulated brush border enzymes including maltase, lactase, alkaline phosphatase and aminopeptidase, but had no effect on sucrase activity. In contrast, IGF-II stimulated lactase and aminopeptidase, but only by the oral route.

Comparative experiments in which the effect of GH, IGF-I and GH plus IGF-I was measured revealed, that all intestinal growth parameters were increased following the administration of IGF-I and GH plus IGF-I, whilst GH alone had no effect (Peterson et al., 1997). These findings are in contrast to in vitro data in which GH was found to significantly increase crypt epithelial cell proliferation in explants of the human small intestine (Challacombe and Wheeler, 1995; Wheeler and Challacombe, 1997). However, this might be attributed to indirect effects of GH as well, mediated by IGF-I.

Additional in vitro studies clearly indicate the mitogenic nature of IGF-I on adult human duodenal mucosa (Wheeler and Challacombe, 1997). The trophic effects of IGF-I to increase crypt epithelial cell proliferation in test explants, exceed those of GH and insulin (Michell et al., 1997a). However, no comparative studies have been conducted in vivo as of yet. As it could be demonstrated that subcutaneous administration of IGF-I improved mucosal structure and absorptive function after small bowel transplantation in rats, the possibility to use IGF-I therapeutically with the aim to improve adaptive changes after surgical resections, has been discussed (Sanderson 1997, Zhang et al., 1995; Chen and Nezu, 1997).

Taken together it can be concluded that there is convincing evidence that IGF-I and other growth factors excreted via milk play an important role in growth and differentiation of gastrointestinal tract tissues and support the concept of a physiological role of colostrum-borne IGFs on the neonate (Baumrucker and Blum, 1993; Fholenhag et al., 1996; Fholenhag et al., 1997)). In addition, clear evidence is provided that orally ingested IGF-I reaches the receptor sites in the gut in its biologically active form.

While the prominent role of IGF-I in the modulation of somatic and gastrointestinal growth in the neonatal was confirmed in several other experiments with rats (Philipps et al., 1997, Steeb et al., 1997, Steeb et al., 1995) and pigs (Burrin et al., 1996), it also became evident, that oral administration of IGF-I results in systemic effects (increase in body weight, liver and brain weight) in suckling rats, and thus indicated the resistance of IGF-I to degradation by gastrointestinal juices of the suckling rat. Radio-labelled IGF-I, when administrated orally remained receptor-active in gastrointestinal tract tissue for at least 30 min post-ingestion (Philipps et al., 1995).

The appearance of IGF-I in mammary secretions has been shown to vary with physiological state. Colostrum of all species contains high concentrations of IGFs when compared with concentrations in mature milk (Baumrucker et al., 1994). This implies that under physiological conditions exposure to high levels of IGF-I occurs only during the short perinatal period. The possible trophic biological effects of a consistent IGF-I exposure via milk throughout the entire life-span needs to be established. Assuming a dose-dependent mitogenic effect of IGF-I, the question remains to be answered, to what extend exogenous IGF-I, being additive to the amount of IGF physiologically present in the gastrointestinal tract (via pancreatic and biliary excretions; Chaurasia et al., 1994), is able to induce any adverse effect as a consequence of long term exposure. This question needs to be addressed as several in vitro studies indicated that IGF-I is mitogenic to several colon carcinoma cell lines (Lahm, 1992; Michell et al., 1997a; Guo et al., 1998)

2.4.1.3. Bio-availability of orally administered IGF-I.

As IGF-I might be important in the treatment of Laron dwarfism and insulin-resistant diabetes, the oral application of recombinant (human) IGF-I has been studied experimentally (the structures of human and bovine IGF are identical). It could be demonstrated that the initial low oral bioavailability of 9.3% could be increased by the co-administration of aprotinin, and, more importantly, by simultaneous application of casein. Casein enhances the oral bio-availability of IGF-I in adult rats to 46% and 67%, respectively (Kimura et al., 1997). The orally administered IGF-I was present in the plasma as the 50-kDa and 150-kDa complexes, indicating that transmucosal transport is facilitated by a specialised transport mechanism.

These data confirm previous experiments, in which an increase of the oral bioavailability of IGF-I in the presence of milk casein had been reported in neonatal calves and neonatal pigs (Xu and Wang, 1996; Vacher et al., 1995),

whereas other studies report a poor absorption rate only (Donovan and Chao, 1997). These experimental data allow the hypothesis that IGF-I possess a considerable oral bioavailability also in humans after consumption of IGF-I enriched milk as the casein acts as inhibitor of several proteases (Playford et al., 1993). This hypothesis needs to be reflected in the light of epidemiological studies indicating a positive correlation between dairy product consumption and breast cancer risk (see section 2.4.2.2.; Del Guidice et al., 1998).

2.4.2. Systemic effects of rBST and IGF-I

2.4.2.1. rBST

Although recombinant BST has been considered "essentially chemically the same as natural bovine growth hormone", certain specific differences are worthwhile to mention:

Recombinant rBSTs differ from the natural growth hormone by 1-9 amino acids. In most cases, the N-terminal alanine is replaced by methionine. Dairy industry experiments indicated that the additional, terminal methionyl residue makes rBSTs more immunogenic (FDA Veterinary Note, 1988).

Short-term studies provided no evidence of carcinogenic properties of rBST in Rhesus monkeys. Although the study design is questionably these data fit into the general concept of species-specificity of peptide hormones. However, the possibility that growth hormone cleavage products might retain certain biological properties including the stimulation of the production of growth factors like IGF-I has never been properly addressed.

Furthermore, the role of other milk constituents, which might be altered in their relative concentration in milk, requires further evaluation as not only milk fat quantity and composition is modified by rBST administration but also an increase in the excreted amount of IGF-I, truncated IGF-I ((des3N-IGF-I) and IGFBPs in bovine milk has been reported (Shimamoto et al, 1992; Groenewegen et al., 1990) (see also section 2.3.2.).

2.4.2.2. IGF-I

Previous epidemiological studies have indicated a positive correlation between dairy product consumption and breast cancer (for review see Outwater et al., 1997).

Detailed analyses on the relative risk (RR) including adjustment of RR coefficients for age at first birth and economic variables provided further evidence that milk and cheese were the only dietary variables to remain significantly positive. It was concluded that the relative risk of breast cancer increases with the amount of dairy products consumed; this trend was not evident with respect to meat consumption.

Hence *in vitro* studies indicated that IGF-I is a potent mitogen for breast cancer cells, the link between milk IGF-I concentrations and the relative risk for human breast cancer was established. This hypothesis is supported by the

fact that human and bovine IGF-I are identical (as mentioned before) and also IGF-I in milk is present in its unbound form.

These mitogenic effects on cell proliferation rate of breast cancer cells could be observed at concentration as low as 1 ng/mL (Zapf et al., 1981). The average concentration in milk varies between 1-34 ng/mL (see section 2.3.2.).

Nearly all breast cancer cell lines and breast cancer cells from fresh tumour biopsies have receptors for IGF-I, and IGF-I binding to both, benign and metastatic human breast tumours is increased compared to normal mammary tissue binding (Macaulay, 1992; Peyrat et al., 1992; Jammes et al., 1992). In addition, highly malignant human breast cancers produce and secrete IGF-I. This observation has been used as diagnostic tool in clinical oncology but also indicates that IGF-I might be directly involved in tumorigenesis. IGF-I causes changes in the cell cycle and activates oncogenes such as *c-fos* (Li et al., 1997). Evidence suggests also that oncogenes may encode IGF-IRs whose over-expression seems to be involved in the transformation from natural mammary tissue growth to breast cancer (Kaleko et al., 1990).

As IGF-I receptors are over-expressed in virtually all breast cancer cell lines they are considered to be related to enhanced proliferation whilst inhibiting programmed cell death (apoptosis). Recently, Resnik et al. (1998) could demonstrate that IGF-IR expression was 14-fold higher in malignant breast tissue than in normal breast tissue and receptor function, as demonstrated by kinase activity, was 2-4 fold higher in purified receptor preparations from malignant breast tissue.

Epidemiological data stressing the role of IGF-I in breast cancer became available with the nested case-control study within the prospective Nurses' Health Study (Hankinson et al., 1998). This well-known study started in 1976 and includes women of different ages (including pre-menopausal and post-menopausal cohorts). Plasma concentrations of IGF-I and IGFBP-3 were measured in blood samples collected in 1989-1990. These IGF-I concentrations were compared by logistic regression with adjustment for other breast cancer risk factors.

A positive relation between circulating IGF-I concentration and risk of breast cancer was found among pre-menopausal women (top *vs* bottom tertile: relative risk 2.33 (1.06 - 5.16) with p for trend 0.08; selecting pre-menopausal women less than 50 years old, the relative risk amounted to 4.58 (1.75 - 12.0) with p for trend 0.02). After adjustment for plasma IGFBP-3 concentrations, the relative risks increased to 2.88 and 7.28, respectively. Neither in post-menopausal women nor among the whole study group, a comparable association between circulating IGF-I concentration and breast cancer could be established (Bohlke et al., 1998).

Del Giudice et al., (1998) found in another case control study a positive association between IGFBP-3, circulating insulin levels and the incidence of

pre-menopausal breast cancer. These recent studies confirm previous case control studies, also reporting a positive relation between plasma IGF-I concentration and breast cancer risk (Bruning and Clemmons., 1995; Peyrat et al., 1993). However, it should be taken into account that the recent studies are also in favour of the suggestion that plasma IGF-I concentrations are an early marker in the identification of women at high risk, rather than indicating a causal relationship between cancer incidence and circulating IGF-I levels. In addition, these epidemiological data suggest a correlation between IGF-I and IGFBP-3, however, the individual contribution to the overall bio-activity in the tissues remains unclear.

Further evidence for the relation between IGF-I and breast cancer originates from experiments with rodent species. Energy restriction can decrease tumour development in multiple models. As energy restriction also lowers IGF-I levels, thereby favouring apoptosis over cell proliferation, energy restriction slows tumour progression. Recent studies (Dunn et al., 1997) confirmed this hypothesis as the protective effect of energy restriction could be abolished by supplementation of IGF-I.

The responsiveness of breast epithelial cells to IGFs is modulated by estrogens and estrogens appear to act at several points of the IGF signal transduction and to regulate both, IGF-I and IGF-II expression as well as IGF binding proteins and type I IGF receptors (Westley et al., 1998; Koval et al., 1998). These data confirm previous studies describing that estrogens increase the level of IGF-I in human breast tissue (Osborne and Arteaga, 1990). Furthermore, IGF-I stimulates estrone sulphatase activity (Purohit et al., 1992) and the number of IGF-I receptors has been found to be positively correlated with the number of estradiol receptors, suggesting synergistic mechanisms (Peyrat et al., 1992).

It is worthwhile to mention that in breast cancer as well as in prostate cancer, bladder tumours, gastric cancer and paraganglioma tumours an increased expression of IGF-II was demonstrated providing further evidence for the role of IGFs in autocrine cancer cell growth *in vivo* (Li et al., 1998).

Finally, IGF-I has been found to be a mitogen for prostate epithelial cells. A prospective case control study of men, participating in the Physician's Health Study revealed a strong positive association between IGF-I levels and prostate cancer risk (Chan et al., 1998; Brower, 1998). Relative risk (RR) varied in an univariate analysis between 0.62 and 4.74 with p for trend of 0.006 (test for linear trend calculated by assigning the medians of the quartiles as scores). Multivariate analysis (with simultaneous adjustment for IGF-I or IGFBP-3) revealed quartiles associated RR values between 0.83 and 10.6 with a p for trend of 0.001.

Again the question remains to be answered whether or not an increased level of circulating IGF-I has to be considered an early marker, predicting prostate cancer risks, rather than indicating a causal association.

3. SECONDARY RISKS RELATED TO THE USE OF rBST IN ANIMAL PRODUCTION

Based on the nature and intrinsic activity of GH in the target animal, a number of secondary effects can be anticipated:

3.1. Effect of rBST on drug metabolism in the target animal species

Growth hormone has been shown to exert its biological effect by regulating the expression of different genes, including the expression of enzymes of the cytochrome P450 family.

Particular reference is made to the CYP2C family, which comprises a considerable percentage of total P450 activities in bovines. CYP2C is involved in the bio-transformation of a wide range of pharmaceuticals facilitating their bio-inactivation and elimination. Down-regulation of CYP2C would result in delayed body clearance and increase the biological half-life of these drugs (Witkamp et al., 1993, Chilliard et al., 1998). This comprises a virtual risk towards an increase of undesirable residues in edible tissues and milk and might lead to an intensified drug residue monitoring.

3.2. rBST and clinical mastitis

The use of rBST might comprise the risk of an increased incidence of mastitis in dairy cows (for a detailed discussion of this item we refer to the corresponding report devoted to Animal Welfare aspects). The public health and food safety aspects of mastitis in dairy cows are exclusively associated with the potential problems of side effects from using antimicrobials in the treatment or prevention of such cases. Treatment of clinical mastitis cases with antimicrobials is not limited to those cases which may be classified as *severe*, although such cases are probably more likely to receive systemic treatment. Also *mild* clinical cases are often treated with local application of antimicrobials, such as the application of formulations for intra-mammary use. Even cases of *sub-clinical mastitis* are sometimes treated with antimicrobials, depending on other factors in the herd, as are cows being dried off before calving (Radostits et al., 1994). The result is that mastitis is the one condition in dairy cows which is associated with use of the largest amount of antimicrobials. It is therefore not surprising, that by far the most frequent reason for residue violations in milk are related to mastitis treatment (Leslie and Keefe 1998). This applies in particular in cases where the principles of Good Clinical Practice are not respected.

The public health reasons for limiting as far as possible the use of antimicrobials in dairy cows are the risk of:

- an increased incidence of allergic reactions from drugs and their metabolites in consumers of milk and dairy products;
- an increased selection of bacteria resistant to antimicrobials.

Allergic reactions:

It is estimated that 3 - 10% of the human population is allergic to penicillin and other beta-lactam antibiotics, which constitute the most common therapeutic treatment for clinical mastitis. There are a few reported cases in the literature on allergic reactions following consumption of contaminated milk.

There is no available data on how the risk of such residues vary with occurrence of mastitis in the source cows, but as a general assumption one may consider that increasing risk of mastitis which is treated by antimicrobials will increase the risk of such residues (Kaneene and Ahl, 1987).

The extent to which this risk is modified or prevented by testing for residues by routine monitoring is also not known, but of course any violation which is detected before the milk is processed will lower the risk of residues in milk for consumption. The test characteristics (sensitivity and specificity) of the test procedures used for detection of residues will, therefore, influence the outcome of the monitoring, and critical evaluations of some of the tests used have been published (Gardner et al. 1996).

Antimicrobial resistance:

The risk of antimicrobial resistance following veterinary, including mastitis related, use of antimicrobials is the subject of another scientific report currently being prepared by the Scientific Steering Committee. Recent publications referring to the specific issue of bacterial resistance following mastitis related use of antimicrobials vary in their evaluation of the phenomenon (Hillerton 1998, Sandgren 1998, Wegener 1998, Aarestrup and Jensen 1999). The issue of antimicrobial resistance in general is subject of several ongoing evaluations in the EU and Codex Alimentarius.

It can be anticipated that with an increase of the incidence of bovine mastitis more veterinary medicinal products will be used. This practice comprises a virtual risk toward an increase of undesirable residues in milk and other edible tissues and might lead to an intensified drug residue monitoring program within the European Community. Furthermore, the increased use of antimicrobial substances in the treatment of rBST related mastitis might lead to the selection of resistant bacteria.

3.3. Adverse effects related to alteration of milk composition

Several reports express concerns about undesirable allergic reactions which might occur after the consumption of milk obtained from rBST-treated cows. Previously, the antibody response to rBST has been investigated as indirect measure of the possible absorption of rBST from the (rat) gastrointestinal tract. However, the question whether or not a change in milk protein composition as a consequence of rBST application to dairy cows might pose an additional risk factor in the development of food allergies has so far not been addressed adequately.

4. SUMMARY AND CONCLUSIONS

Numerous reports have indicated that the application of recombinant growth hormones (rBST, rbST) increases productivity of dairy cows measured as total milk yield per animal per lactation period. The application of rBST therefore may result in economic benefits although no therapeutic indications have been considered in the target animal species to date.

Based on its peptide nature, rBST has to be applied parenterally and the concept of species - specificity implies that residual amounts of unchanged rBST fail to induce a biological response in species (including humans) other than bovines. However, the nature of rBST cleavage products and their biological activity has not been investigated in detail.

Comparably to the endogenous growth hormone, rBST is known to increase the level of circulating IGF-I in the target animal followed by an increased excretion of IGF-I in milk. Consequently increased levels of IGF-I in milk have to be included in the estimation of potential health hazards originating from the zootechnical use of rBST.

IGF-I is a physiological constituent of bovine milk. Data on the actual amount of IGF-I in milk are inconsistent as physiological levels show a considerable variation depending on the age of the animals, state of lactation and nutritional status. The highest IGF-concentrations in milk are found at the initial phase of lactation (colostrum) and decline as lactation progresses.

The various analytical techniques for the determination of IGF-I and its truncated forms need to be evaluated in validated procedures. Present data do not provide a conclusive answer to whether or not previously applied analytical techniques have underestimated the actual IGF-I level in milk by neglecting the protein-bound fraction, and to what extent the ratio between free and bound IGF-I in milk has changed as a consequence of rBST treatment resulting in a relative increase of the free IGF-I fraction.

Application of rBST increases the amount of excreted IGF-I in milk by 25-70 % in individual animals. The Committee noted that bovine milk may contain truncated IGF-I (des(1-3)IGF-I) which was found to be even more potent than IGF-I in the anabolic response when given subcutaneously to rats. No quantitative data are available indicating the additional level of this truncated form of IGF-I in milk from rBST-injected dairy cows.

The biological activity of IGF-I comprises endocrine, paracrine and autocrine effects and IGF-I has been identified as cellular growth factor with mitogenic, anti-apoptotic properties and may thus directly interfere with physiological mechanisms involved in the removal of transformed cells. Evidence on the physiological essentiality of IGF-I in foetal and perinatal development is accumulating. Biomedical research focuses on the possible use of IGF-I in the therapy of distinct diseases, whereas the detrimental role of IGF-I in tumour progression is disputed.

Experimental evidence for an association between IGF-I and breast and prostate cancer is supported by epidemiological studies. The bimodal activity of IGF-I being

essential in the process of cellular differentiation regulating the expression of several genes, and acting as cellular growth factor with anti-apoptotic properties hinders the definition and establishment of a no-adverse-effect level, a paradigm in conventional risk assessment.

Advocates of the medical (therapeutic) use of IGF-I refer to the short half-life and the auto-regulatory mechanisms sequestering free biologically active IGF-I via endogenous binding proteins (IGFBPs).

Opponents refer to the epidemiological evidence arising from the recently published cohort studies indicating an association between circulating IGF-I levels and the relative risk of breast and prostate cancer, respectively.

Elevated plasma IGF-levels may be considered as a predictive marker for breast and prostate cancer. However, it should be emphasised that all these epidemiological studies refer to a time interval in which exposure to dairy products originated exclusively from non-rBST treated animals. Whether or not the use of rBST will modify the level of risk, remains to be substantiated.

Following the globally accepted concept of risk assessment it is concluded that:

- Direct risks associated with the use of rBST in dairy cows appear to be related to the possible increase of IGF-I levels in milk. The diverse biological effects attributable to the intrinsic activity of IGF-I, exerting a broad variety of metabolic responses through endocrine, paracrine and autocrine mechanisms, make the definition of an in vivo quantitative dose-effect relationship virtually impossible.

- Risk characterisation has pointed to an association between circulating IGF-I levels and an increased relative risk of breast and prostate cancer. In addition, the possible contribution of life span exposure towards dietary IGF-I and related proteins, present in milk from rBST treated cows, to gut pathophysiology particularly of infants, and to gut associated cancers need to be evaluated.

- The available data basis for exposure assessment, i.e. the amount of IGF-I and/or its truncated forms excreted in milk following the administration of rBST to dairy cows, is incomplete.

In addition secondary risks associated with the use of rBST in dairy cows are:

- Potential changes in milk protein composition which might favour allergic reactions.

- An increased use of antimicrobial substances in the treatment of rBST related mastitis which might lead to an increased risk of residue formation in milk and to the selection of resistant bacteria.

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The members of the Scientific Committees listed hereunder can be contacted through the Secretariat of each Committee, whose whereabouts figure on this page.

Final List of Members of the Scientific Committees, as nominated by the Commission on 4/11/97, will appear in the OJ of 11/11/97

Alphabetical list of the scientists appointed by the Commission as members of the Scientific Committees set up by Decision 97/579/EC of 23 July 1997.

Prof. Osterhaus, Albert Professor, Erasmus Universiteit Rotterdam, Faculteit der geneeskunde en gezondheidswetenschappen, Instituut voor Virologie, Nederland - *President of the Committee*

Prof. Caporale, Vincenzo Direttore, Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Italia

Prof. Dr. Fink-Gremmels, Johanna Professor, Universiteit Utrecht, Afdeling Veterinaire Farmacology, Farmacie en Toxicologie, Dept. Veterinaire Basiswetenschappen -Faculteit Diergeneeskunde, Nederland

Dr. Gilbert, Richard J. Director of Food Hygiene Laboratory and Deputy Director PHLS, Central Public Health Laboratory PHLS, Food Hygiene Laboratory, , United Kingdom

DVetMed Johnston, Alexander M. Senior Clinical Tutor, University of London, The Royal Veterinary College, Department of Farm Animal and Equine Medicine and Surgery, United Kingdom

Prof. Jouve, Jean-Louis R. Prof., Chef D'Unité, Ecole National Vétérinaire de Nantes, Unité Hygiène et Qualité des Aliments, France

Prof. Dr. Kaaden, Oskar-Rüger Institutsvorstand, Ludwig-Maximilians-Universität-München, , Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin, Deutschland

Professore Macrì, Agostino Direttore, , Istituto Superiore di Sanita (ISS), Laboratorio di Medicina Veterinaria (MVE), Italia

Prof. Dr. Mantis, Antonios J. Professor, Rector of University untill august 1997, Aristotle University of Thessaloniki, School of Veterinary Medicine, Department of Food Hygiene and Technology, Greece

Prof. DVM Nurmi, Esko Professor, former Director General, National Veterinary and Food Research Institute (EELA), Finland

Profesor Quinto Fernandez, Emiliano J. Profesor Titular de higiene e inspeccion de los alimentos, Universidad Autonoma de Barcelona, Facultad de Veterinaria, Departament de Patologia i de Produccio Animals, España

Ph.D. Schlundt, Jorgen Head of Section, National Food Agency of Denmark, Institute of Toxicology, Section of Microbiology, Denmark

PhD Vagsholm, Ivar Head of Centre, National Veterinary Institute, Zoonosis Centre, Sverige

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ProF Dr. van Knapen, Frans Professor, Universiteit Utrecht, Faculteit Diergeneeskunde, Hoofdafdeling Voedingsmiddelen van Dierlijke Oorsprong, Nederland

Dr. Vuitton, Dominique A. Professeur en Immunologie Clinique, Université de Franche-Comptć, Faculté de médecine et de Pharmacie, France

Prof. DVM Willeberg, Preben Professor, The Royal Veterinary and Agricultural University, Animal Science and Animal Health- Division Ethology and Health, Denmark

[DI - [CONSUMER HEALTH PROTECTION] - [SCIENTIFIC COMMITTEES] - [SCIENTIFIC COMMITTEE ON VETERINARY MEASURES RELATING TO PUBLIC HEALTH]

UNIVERSITY OF ILLINOIS
AT CHICAGO
Dr. Samuel S. Epstein
UIC Office of the Dean (MC 922)
School of Public Health
2121 West Taylor Street
Chicago, Illinois 60612-7260

Food and Drug Administration
Department of Health and Human
Services
Room 1061, 5630 Fishers Lane,
HFA-305
Rockville, MD 20852